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of

FOREST RESEARCH ORGANISATIONS

Working party: S2. 01. 06

SEED PROBLEMS

PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM ON FOREST TREE SEED STORAGE

Petawawa National Forestry Institute
Chalk River, Ontario, Canada
September 23-27, 1980

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Proceedings of the International Symposium on Forest Tree Seed Storage

September 23-27, 1980
Petawawa National Forestry Institute,
Chalk River, Ontario, Canada, K0J 1J0

Compiled and Edited
by
B.S.P. Wang and J.A. Pitel

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Organizing Committee:

Canadian Forestry Service

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- Willard H. Fogal
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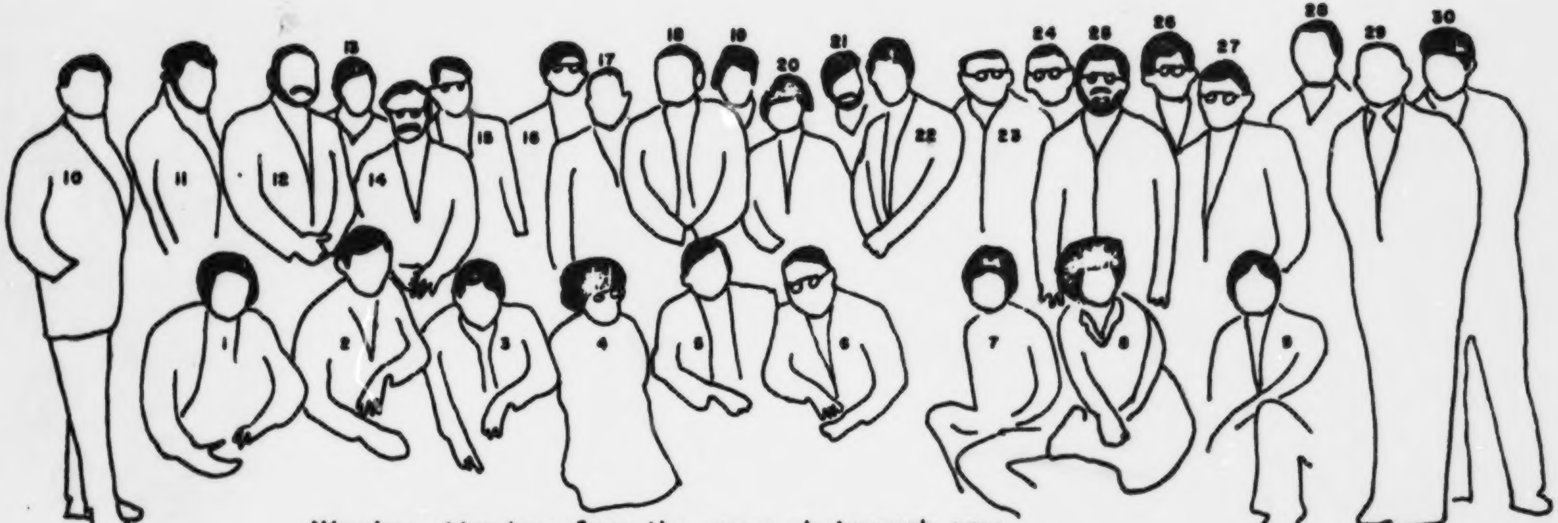
- E. Kris Morgenstern
- Jack A. Pitel
- Ben S.P. Wang (chairman)
- Dave A. Winston
- Kit W. Yeatman

Ontario Ministry of Natural Resources

- Douglas A. Skeates

LIST OF IUFRO ATTENDEES

1. Yasuomi Tanaka
2. George Edwards
3. Krishan Kamra
4. Claudine Muller
5. Sandy Robertson
6. Ben Wang
7. Rose Marie Rauter
8. Carole Leadem
9. Domingo Chai
10. Dal Travnick
11. Fred Haavisto
12. Doug Skeates
13. Wim Vanborrendam
14. Jack Pitel
15. Kit Yeatman
16. Dave Winston
17. Ray Latimer
18. Milan Simak
19. Mark Campbell
20. Alena Straka
21. Ron Smith
22. Rob Bowden-Green
23. Dick Altmann
24. Hugh Schooley
25. Brian Swaile
26. Brian Haddon
27. Boleslaw Suszka
28. Willard Fogal
29. Raj Prasad
30. Bill Baker



Missing attendees from the group photograph are:

Stanley B. Carpenter
Armand Corriveau
Yves Lamontagne
Walter Trowsdale
Moe Anderson
Ernie Gilchrist
Garry Scheer
Francine Ackerman

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PREFACE

During the Second IUFRO International Symposium on the Physiology of Seed Germination (Working Party S2.01.06) held in Japan, 1976, participating members at the business session approved the formation of an Editorial Board and recognized the necessity for some editing of future symposium proceedings. Based on this decision, papers presented at or sent to this symposium on Forest Tree Seed Storage at Petawawa National Forestry Institute have been reviewed by at least one referee, together with some minimum editing by the editors. This effort has proved to be beneficial as many major and minor questions and ambiguous expressions have been corrected or clarified for better reading. The submitted texts have been retyped so that a more standardized format is obtained, such as a uniform method of presenting references, species names are in italics and the same type size and style are used. Also a French translation of the Abstract is included that was not present in the original version by the authors. However, the authors are responsible for major editorial matters and also for the scientific content.

The proceedings are published in the same manner as arranged in the Symposium Program. The twenty-four papers are grouped into the following four Technical Sessions: (1) General problems and approaches to tree seed storage; (2) The effects of cone storage conditions on seed; (3) Effect of insects and diseases on cone and seed storage; and (4) Factors affecting seed storage. These sessions are identified by a title page and the names of the session chairperson and co-chairperson.

The proceedings also include field tour information, addresses by the Working Party Chairman, host representatives, organizing committee and tour guides' reports, as well as the Working Party's research project reports. The editors wish to thank the Canadian Forestry Service for sponsoring the symposium and publishing the proceedings; and the Petawawa National Forestry Institute for providing the facilities and resources for hosting the symposium and subsequent handling and typing of the proceedings.

B.S.P. Wang
J.A. Pitel

WELCOME SPEECH by B.S.P. Wang

Ladies and gentlemen, on behalf of the Organizing Committee, I would like to welcome you all to the IUFRO International Symposium on Forest Tree Seed Storage at Petawawa.

Since very few of you know how this symposium came about, I would beg your indulgence to report to you very briefly.

It was at the 1976 IUFRO Symposium in Japan that the Canadian Forestry Service offered to host this symposium, and the subject of the symposium on tree seed storage was discussed and accepted by the attending participants.

Since then there was a lot of interest expressed by many people from different countries. When the Symposium was officially announced in international journal and newsletter media and by mail invitations, we received a very enthusiastic response from many seed workers. In the pre-registration we had close to 50 interested participants from over 22 different countries. However, when the final registration was sent out, the returns were greatly reduced, primarily due to financial difficulties and curtailed travelling abroad. Thus, as you can see here, the attendance at the symposium is fewer than we expected. However, we should consider ourselves fortunate to be able to participate in the symposium, especially those who came from countries like Sweden, Poland, France and as far as Malaysia.

Perhaps the Working Party should consider different approaches to future symposia or workshops. This matter may warrant further discussion during the Working Party's business sessions.

We chose this time of the year to hold the symposium because we would like you to see our beautiful eastern Canadian fall colour. Although it has not reached its full development yet, I guarantee you that you will see a lot more before the close of this symposium.

I would like to take this opportunity to thank members of the Organizing Committee, our Ottawa HQ's, the Acting Director and the administration staff of our Institute and the Working Party Chairman and Co-chairman, the Ontario Ministry of Natural Resources staff; it would not have been possible to get the symposium ready without their valuable assistance and dedication.

During the 5 day symposium, we will try our best to make your attendance at the symposium and your stay around our area as comfortable and enjoyable as we can.

WELCOME SPEECH by Mr. R.F. Ackerman, Acting Director, Petawawa National Forestry Institute.

Mr. Chairman, Ladies and Gentlemen, on behalf of the Canadian Forestry Service and the Petawawa National Forestry Institute, I would like to welcome you to Canada and to this Institute. Canada is a forestry country, but many Canadians are not aware of the great value of their forests and they do not consider this vast renewable resource a source of national pride. However, this attitude is changing rapidly and the governments are becoming more interested in forest management. There is an urgent need to renew the vast areas of forest land which have been logged and degraded. Tree seed storage is important for solving the forest renewal problem. At this Institute many programs are being carried out to help solve some of the particular forest management problems.

Finally, I wish that you all have a very successful symposium.

WELCOME SPEECH by the Working Party Chairman,
Professor M. Simak (Sweden)

Mr. Acting Director Ackerman,

Ladies and gentlemen.

It is an honour and great pleasure for me to welcome you at this fourth IUFRO-symposium on Seed Storage, organized by the Working Party on Seed Problems.

On behalf of IUFRO and in the name of all the participants I wish to express our gratitude to the Canadian Forestry Service and to the Petawawa National Forestry Institute, which have with great willingness and generosity supported the realization of this symposium.

All the work associated with organizing this symposium was carried out by our friend Ben Wang and his organizing committee. We all know very well how much time and effort has to be sacrified to prepare such an event, and we therefore heartily thank you Ben and all your associates for your excellent job. Ben, we all here will do our best to make this symposium successful, so hopefully you will get full satisfaction for your hard work in arranging this meeting of our Working Party.

Now let me say a few words about the subject of this symposium:

Seed storage has been practised by man for several thousand years. Archeological excavations showed that man stored different agricultural seeds in clay pots beneath the earth in order to build up food reserves for his family or to secure seeds for the next sowing season. As against this old agricultural practice, the preservation of forest seeds is a very young discipline which started about 100 years ago. At this time an intensive collection of forest seeds was needed for artificial regeneration being introduced into forestry. Herewith arose the need to store the seeds for several years ahead in order to secure a continuous seed supply from year to year.

In the beginning, the storage technique of forest seeds was rather primitive, which reflected itself in the poor plant production received from the improperly stored seeds. The seed storage conditions were improved in the last half of this century mainly thanks to the development of new technical facilities, for instance, storage rooms with controlled temperature and instruments for controlling the seed humidity, etc. During this period when the main interest was paid to the ecological conditions of the stored seeds the effect of the storage was measured mainly by the germinability which the seeds showed at the end of the storage period.

At present, we are more and more penetrating into the root of the storage problem through analyses of the biochemical, physiological, genetical and other processes occurring in the seeds during the preservation. Consequently, not only the germinability itself but also other properties of the stored seeds are considered when looking for the best storage conditions of a particular seed specimen. I think that this dialectic approach to seed storage problems is very necessary to carry out this work successfully. It would therefore be desirable to consider this broad view on the storage of forest seeds in the coming discussions of this symposium.

There are two practical problems concerning storage of forest seeds, which could be particularly interesting as projects for our Working Party.

Firstly, many new tree species are being introduced in forestry, for example those in the tropical countries. Unfortunately, our knowledge about seed storability of these species is limited. Judging from the varying seed biology of these species, it is probable that these seeds will also require different storage conditions. Another group of seeds are the s.c. recalcitrant seeds, which earlier were considered practically as not storable. As many of the tree species with recalcitrant seeds became useful in forestry, the interest to study the storage conditions of these seeds increased. Hence, we have here an embryo for a new common project which can be included in the future programme of our Working Party.

Secondly, in recent years gene banks have been founded in which the valuable reproductive forest material - mainly seeds - will be preserved for many years. The idea of the gene bank is undoubtedly very useful, however, when storing the seeds in the seed banks, we have to remember that even under the most sophisticated storage conditions, the seeds are exposed to genetic changes during a long storage period. After all, a prolonged artificial preservation of the seeds is something un-natural, as the adaption of the seeds for long-term storage was not considered in the evolution process of Nature. Consequently, a long-term storage can pardoxically work against the idea of a gene bank by changing the genetic constitution of the stored seeds. Such undesirable changes can occur more frequently in forest tree seeds, which usually have to be stored for a longer time than the agricultural seeds. I think we should pay more attention to these genetic effects of long-term storage and be careful when applying the experience from agricultural research to forestry. Perhaps a project on this subject to be carried out by our Working Party would be of great benefit for the seed bank idea.

Before I finish this introduction, I would like to remind you that next year will conclude the second 4-year period of the existance of our Working Party. It was at the 1973 IUFRO congress in Gainsville, USA that our Working Party was established. Since then, the seed problems has become an important subject in the great family of the numerous other Working Parties within the IUFRO. Since this time, our Working Party has developed a lively activity, witnessed by the seed symposia held at Bergen, Norway; Tokyo, Japan; Starkville, USA; and now this at Petawawa. Our Working Party has also organized in cooperation with the Forest Tree Seed Committee of ISTA, workshops at Gilford, Great Britain; Macon, USA; and now a forthcoming workshop at Chetumal, Mexico. Many of our members were and are very active in performing successful international projects of great value for seed research.

Well, you see, that the little seed symbolising our Working Party which was sown at Gainsville seven years ago, has now grown to a huge tree with strong branches representing the different activities of our Working Party. We are going to discuss during this symposium, the future organization of our Working Party. I hope that all of you will actively contribute your ideas and proposals during these discussions. We, the seed workers, all need a prosperous, strong Working Party on seed problems which will be a dignified forum for forest tree seed research of the world and where the actual seed problems can be discussed at any time.

Herewith I open the 4th Symposium on seed storage of the IUFRO - W.P. S2.01.06.

TECHNICAL SESSION 1: GENERAL PROBLEMS AND APPROACHES TO TREE SEED STORAGE

Chairperson: B. Suszka
Co-chairperson: C. Leadem

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[illegible]

[illegible]

[illegible]

[illegible]

[illegible] Working Party. [illegible]

[illegible]

UNDERGROUND SEED COLD STORAGE FACILITIES

D. Altmann and L. Lafleur [1]
Pine Ridge Forest Nursery, Smoky Lake, Alberta, Canada

ABSTRACT

The design of the seed cold storage facilities at Pine Ridge Forest Nursery is based on the concept of root cellars used by early settlers in the prairie provinces. Storing seed underground achieves a certain amount of natural temperature and humidity control due to a minimum of exposure to drastic weather changes.

RÉSUMÉ

Les entrepôts frigorifiques des graines de la pépinière forestière de Pine Ridge sont construits sur le principe des caves à légumes employées par les premiers colons dans les Prairies. Un entreposage souterrain des graines procure une certaine régulation naturelle de la température et de l'humidité, du fait d'une exposition minimale aux variations importantes de la température.

---

[1] Respectively Production Research Forester and Supervisor Seed Program, Pine Ridge Forest Nursery, P.O. Box 750, Smoky Lake, Alberta, Canada

INTRODUCTION

Pine Ridge Forest Nursery processes and stores coniferous tree seed required for reforestation of Crown land by the Alberta Forest Service and forest industry in Alberta.

A separate fire-proof concrete building was constructed to store a minimum of 50,000 kilograms of white spruce and lodgepole pine seed in four individually controlled freezer compartments.

Seedlots are kept separate by provenance.

RATIONALE OF UNDERGROUND STORAGE

The concept of building cold storage facilities underground has several major advantages.

One of these is almost full protection against destruction by fire of the total long-term supply of tree seed available for reforestation in Alberta.

Another advantage is a considerable saving in energy requirements. Air temperatures at Pine Ridge Forest Nursery can vary during the year from extremes of -50°C to +35°C. The constant environment located 2 metres underground allows for the use of smaller refrigeration units and eliminates the need for heating to maintain a constant temperature in the freezer compartments. Power used for the entire seed cold storage facilities is approximately 450 to 470 kilowatt hours per day (costing $9 to $10 per day) because the freezer units need only to be cooled from +4°C to -18°C.

The natural insulation of soil around the building is a built-in protection in case of a power failure or major breakdown of equipment because the freezers warm up very slowly when shut down. During a mechanical failure the temperature inside the freezer compartments increased only 10°C in 72 hours.

Underground storage also eliminates costs of exterior maintenance of the building.

A disadvantage of locating the building underground is the problem of keeping the entrance ramp free of ice buildup during the winter, but this is easily solved by prompt clearing of snow.

LOCATION AND CONSTRUCTION OF THE BUILDING

In order to minimize the problems of drainage and to lessen the earth moving requirements, a site was selected where a natural hillside was available for excavation. The hill was removed, the reinforced 43 cm thick concrete walls were erected and the prefabricated freezers installed before the roof was put in place. After the completion of the building the hill was put back so that all except the entrance ramp and top of the mechanical room are buried beneath a minimum of 2 metres of overburden.

The total cost of the seed cold storage facilities, including all equipment costs, was slightly over 550,000 dollars in 1978.

DESCRIPTION OF SEED COLD STORAGE FACILITIES

Ante Room

At the bottom of the entrance ramp a set of double doors opens into an ante room 4 m wide by 3 m long. This ante room is a buffer zone to protect the handling room from the outside elements. The ante room is maintained at approximately 18°C by a small electric heater.

Handling Room

Another set of double doors leads from the ante room into the handling room which is 4 m wide and 8 m long. This room is maintained at +4°C (which is the approximate underground temperature) so that seed being withdrawn from cold storage does not pass directly into a warm environment which could cause condensation on the seed when the storage containers are opened.

The handling room is used as a working area for taking samples of seed in storage, performing other necessary checks, or withdrawing seed from storage containers.

Freezer Units

Four Bally freezer units open into the handling room. These are prefabricated commercial freezers measuring 6.14 m x 6.53 m x 4.37 m high and constructed of a galvanized steel sandwich with approximately 90 mm of insulation between the layers of steel. The units are free standing with an air space of 10 cm between the exterior walls of the units and the walls of the building. The doors opening into the handling room are a pair of rubber impact swinging doors (2.1 m high and 1.5 m wide) covered on the outside by a sliding insulated galvanized door (2.1 m high and 1.8 m wide).

Seed is stored in these four freezers at a temperature of -18°C and a moisture content of between 5 and 8%. The relative humidity in the freezers is maintained at somewhat less than 50%. Shelving in the units was designed in conjunction with the design of seed storage boxes and shelf loading methods. The shelving structure is constructed of 76 mm x 51 mm angle Dexion uprights and 51 mm x 51 mm angle Dexion crossmembers with 9 mm K3 particle board shelving. Each shelf holds 120 kg of tree seed in either six 20 kg boxes or twelve 10 kg boxes, giving a storage capacity for each freezer of 15,120 kg of seed. Total storage capacity for the entire underground freezer is 60,480 kg of seed. Sufficient seed can be stored to handle more than ten years of direct seeding

Seed at Pine Ridge Forest Nursery is stored in 5 mil thick plastic bags with gussets to fit a square box and hold 10 kg of seed each. The bags are heat sealed to minimize moisture transfer and are placed into either 10 kg or 20 kg capacity waxed cardboard storage boxes. The boxes consist of a bottom of single-layered waxed cardboard sides and double-layered bottom and a top of similar design which slides over the bottom resulting in a storage container having two layers of waxed corrugated cardboard on all sides. The outside of the box is labeled Pine Ridge Forest Nursery and has a space for entering the seedlot number and quantity of seed enclosed. The 20 kg capacity boxes measure 360 mm x 360 mm x 430 mm high while the 10 kg capacity boxes are only 215 mm high. On the shelves the large boxes are placed in one layer while two small boxes occupy as much space as a large one. The shelves are loaded manually with the assistance of an electric-lift manual pallet jack and four sets of rolling stairs. Aisles were designed to allow easy movement of this equipment.

Mechanical Room

A stairwell at the far end of the handling room leads upstairs to the mechanical room and the emergency exit. The mechanical room is located directly above the handling and ante rooms and houses two refrigeration units for the handling room and two freezer units for each Bally freezer as well as electrical panels, temperature and humidity recorders and the alarm system.

The handling room as well as each freezer is temperature controlled by a single refrigeration unit while a second unit for each is on standby in case of a mechanical breakdown or maintenance requiring shut down of a unit. An alarm system consisting of an internal buzzer and flashing light as well as an externally mounted red flashing light warns of abnormal temperature fluctuation or compressor failure. Four recorders provide a permanent record of temperature and relative humidity in the freezers.

The mechanical room is temperature controlled to provide a constant environment for the air-cooled units. This is achieved by a series of four roof fans, two sets of louvers and two small electric heaters hooked into a step-staging temperature control panel. For easy removal of compressors requiring outside maintenance or replacement, a track and chain hoist runs above the line of compressors and a trap door is located in the floor directly above the entrance ramp outside the ante room entrance.

Specifications of some equipment are listed in the Appendix.

CONCLUSION

After nearly two years of operation the design of the seed cold storage facilities proved to be effective and reliable. More than 40,000 kg of white spruce and lodgepole pine seed are currently in storage.

APPENDIX

Freezers

Bally - Model #6084-5

Manufactured by:
Bally Refrigeration of Canada Ltd.,
Brockville, Ontario
K6V 5V1

Cooling Unit for Handling Room

Keeprite Condensing Unit - Model CCUO 2MA
- air cooled

Manufactured by:
Keeprite Products Ltd.
Brantford, Ontario

Carlyle Compressor - Model 06DM - 8080 GA0420

Manufactured by:
Carlyle Compressor Co.,
U.S.A.

Freezing Units for Freezers

Keeprite Condensing Unit - Model CCUO 2LA
- air cooled

Carlyle Compressor - Model 06D - 1090 GA0400

SEED STORAGE IN ONTARIO

R.B. Latimer

Ontario Tree Seed Plant
Ontario Ministry of Natural Resources, Angus, Ontario L0M 1B0 Canada

D.A. Skeates

Ontario Ministry of Natural Resources
Ontario Forest Research Centre, Maple, Ontario L0J 1E0 Canada

ABSTRACT

The Ontario Tree Seed Plant was established in 1922 to maintain a supply of seed for a one million tree planting program. Changes in storage conditions and magnitude of program are described. Currently 75 species are maintained in frozen storage at -3°C to support the provincial reforestation program, including forest re-establishment, ornamentals and species required for wildlife habitat management.

RÉSUMÉ

L'Ontario Tree Seed Plant a été établi en 1922 pour maintenir un approvisionnement de graines en vue d'un programme de plantation d'un million d'arbres. Des modifications des conditions de stockage et de l'amplitude du programme sont décrites. À l'heure actuelle, 75 espèces sont conservées à l'état congelé à -3°C pour subvenir aux besoins du programme provincial de reboisement, ce qui inclut la reconstitution des forêts et la plantation des arbres d'ornement et des essences nécessaires à l'aménagement d'habitats de la faune.

When loggers were cutting vast quantities of pine from the sand plains of Ontario's Simcoe County, the supply must have appeared limitless. A.H. Richardson (1929) described the harvesting of white pine of 1.2 metres, and red pine of .9 metres diameter on the stamp. The village of Angus, where the Ministry of Natural Resources has its seed extraction facility, was the shipping centre for 17 sawmills, with 10-12 trainloads of lumber and squared timber leaving for the south weekly. He noted the use of 12 teams of horses hauling a single white pine mast 36 metres long.

Richardson needed no apology for his comments on forest exploitation. "It may seem a long look back to virgin stands of timber which once surrounded this settlement. It is a long look into the future when this timber will be put back again in all its value and beauty by modern reforestation methods, but the important thing is this: these stands of timber need never have been cut in their entirety. By wise management and the exercise of scientific forestry knowledge, many mills might still be operating in Angus, and the village still be a prosperous settlement." The wasteful exploitation, so well demonstrated at Angus and in many other places in Ontario is nothing new in world history. It has resulted in Ontario foresters being in the constant position our baseball friends would describe as having to play 'catch-up ball'.

The first significant step taken in catching up was the initiation of red and white pine cone collection in camps in the Angus area in 1920 to 1922. Cones were stored and processed in army buildings at nearby Camp Borden. In 1923, 3.3 hectares of land was purchased and the Ontario Tree Seed Plant established to supply seed for production of one million trees annually between the first two provincial nurseries, Midhurst near Angus, and St. Williams near Lake Erie. Richardson (1934) described the seed storage facility. "The seed at Angus is stored in an underground vault 10 feet (3 metres) wide and 40 feet (12.2 metres) long and has a capacity of 10-15,000 pounds (4500-6800 kg) of seed. The temperature in this vault is recorded the year round by a recording thermometer and the fluctuation is about 22° (12°C) degrees, reaching a temperature of 62 degrees F (17°C) in the summer and a temperature of 40°F (5°C) in the winter."

In 1933 a refrigerated vault was built providing year round storage at 5°C (Latimer 1973). Seed of some species could be held up to 10 years or more without too great a loss in germination. The moisture content in storage was maintained at 6% in large sealed glass containers. The facility consisted of 2 rooms, each 5 metres square, with a total shelf space for 1030 jars, each of 23 litre capacity, or close to 12,000 kg of seed.

The present seed storage facility was constructed in 1968-69. The old cold storage vault was converted to a freezer. A constant temperature of -3°C is maintained year round. The 185 square metres of shelf space will hold 2351 plactic containers, each with a capacity of 12.12 kg, or a total of 28,500 kg of seed. The containers were a modification of the Wajax back pack designed at Angus.

Annual provincial seed needs rose from 300 million viable seeds in 1957 to 700 million in 1973. Currently the province is silviculturally treating 80,000 hectares of annual cut over. By, 1985 the stated intent is to regenerate the complete 195,000 hectares cut over annually, that is treatment of 135,000 hectares in addition to 60,000 hectares expected to regenerate naturally. This means almost doubling our silvicultural effort which by extrapolation should require

1.2 billion viable seeds annually. However, much of this additional treatment will require extensive management, i.e., direct seeding for which vastly more seed is required. One scientist has estimated we may require up to 8 billion viable black spruce seeds alone to seed the black spruce upland sites. This would represent 54% of the black spruce cover type cut over annually.

The problem of storing adequate seed supplies for this program is made even more difficult by the change in seed policy in 1978 (Skeates 1979). Since 1963, seed has been bulked and stored by seed zone adapted from ecological site regions described by G.A. Hills (1960). These regions were very broad and were used in the absence of any knowledge of population variation. It was realized that movement of seed over the length and breadth of these large zones carried with it inherent potential growth losses. Currently seed zones are subdivided by administrative districts in northern Ontario, or administrative regions in the three smaller southernmost regions. The identity of an ever increasing number of registered stands must also be maintained in storage, resulting in numerous small seed lots which make inefficient use of standard storage containers. However, none of these problems is considered insurmountable with the present facility.

Seed is maintained in storage for three basic programs in the Ministry. By far the greatest quantitiy is for the provincial silvicultural program involving storage of 21 species (Table 1). Of this 22,542 kg or 4.2 billion viable seeds are accounted for by 5 coniferous species (red, white and jack pine and white and black spruce), and well over half of this is accounted for by jack pine required for direct sowing in northern Ontario.

Table 1. Seed in Storage for Standard Reforestation Stock Program, June 1, 1980 at Ontario Tree Seed Plant

Conifers		Kg	Viable Seeds	
Pinus strobus	White Pine	4,872	208	Million
Pinus resinosa	Red Pine	2,844	208	
Pinus sylvestris	Scots Pine	67	8	
Pinus banksiana	Jack Pine	12,059	2,396	
Picea mariana	Black Spruce	751	696	
Picea glauca	White Spruce	2,016	713	
Picea rubens	Red Spruce	1	.4	
Picea abies	Norway Spruce	322	33	
Thuja occidentalis	White Cedar	177	98	
Juniperus virginiana	Red Cedar	1	.1	
Larix decidua	European Larch	-	-	Samples
Larix leptolepis	Japanese Larch	26	3	
Pinus monticola	Western White Pine	-	-	2 Kg. Low Viab.
Hardwoods				
Acer saccharum	Sugar Maple	2	75	Thousand
Fraxinus pennsylvanica	Green Ash	< 1	33	
Robinia pseudoacacia	Black Locust	294	12,448	
Betula alleghaniensis	Yellow Birch	27	5,891	
Betula papyrifera	White Birch	9	8,849	
Tilia americana	Basswood	523	3,874	
Quercus rubra	Red Oak			14 Kg. Low Viab.
Prunus serotina	Black Cherry	144	777	

Table 2. **Seed in Storage for Ontario Land Act Program*, June 1, 1980 at Ontario Tree Seed Plant**

Conifers		Kg	Viable Seeds	
Picea pungens	Blue Spruce	< 1	142	Thousand
Pinus nigra	Austrian Pine	32	1,147	
Pinus mugo	Mugho Pine	18	1,735	
Abies balsamifera	Balsam Fir	4		
Abies koreana	Korean Fir	2	28	
Hardwoods				
Acer ginnala	Amur Maple	< 1	126	
Acer negundo	Manitoba Maple			Samples, Low Viab.
Betula pendula	European White Birch		5	Samples, Low Viab.
Caragana arborescens	Caragana (Pea Tree)	18	618	
Tilia cordata	Small-leaf Linden	30	1,016	
Tilia platyphyllos	Large-leaf Linden	5	44	
Ulmus pumila	Chinese Elm	7	328	
Magnolia stellata	Magnolia			Samples
Physocarpus opulifolius	Ninebark	2	1,120	
Gymnocladus dioicus	Kentucky Coffee Tree	6	3	
Liriodendron tulipifera	Tulip-tree	2	33	
Cercis canadensis	Redbud		4	
Platanus occidentalis	Sycamore	4	212	
Ptelea trifoliata	Hop Tree	12	75	
Sorbus aria	White Beam Mtn. Ash	1	7	
Sorbus americana	American Mtn. Ash	< 1	62	
Sorbus aucuparia	European Mtn. Ash	5	363	
Alnus glutinosa	European Alder	11	459	
Gleditsia triacanthos	Thornless Honey Locust	49	238	
Lonicera tatarica	Honeysuckle	22	1,175	
Catalpa speciosa	Catalpa (Bean Tree)	1	60	
Elaeagnus angustifolia	Russian Olive	169	1,411	
Elaeagnus commutata	Autumn Olive	146	3,943	

*Ontario Land Act, Species for Highways, Provincial Institutional Planting, etc.

Table 3. Seed in Storage for Wildlife Program*, June 1, 1980 at Ontario Tree Seed Plant

		Kg	Viable Seeds	
Amelanchier canadensis	Juneberry	< 1	21	Thousand
Aronia melanocarpa		< 1	15	
Cedrus deodora				1 Kg Low Viab.
Chaenomeles japonica				1 Kg Low Viab.
Cornus racemosa	Panicled Dogwood			< 1 Kg Low Viab.
Cornus stolonifera	Red Osier Dogwood			118 Kg Low Viab.
Cornus florida	Eastern Flowering Dogwood			17 Kg Low Viab.
Cornus canadensis	Bunchberry	< 1	11	
Lespedeza bicolor		< 1	76	< 1 Kg Low Viab.
Malus baccata	Siberian Crab Apple		8	1 Kg Low Viab.
Rosa multiflora	Multiflora Rose	102	11,584	
Pyrocanthus rosedale				1 Kg Low Viab.
Viburnum lentago	Nannyberry	118	985	
Viburnum cassinoides	Wild Raisin	25	844	
Viburnum opulus	European High Bush Cranberry	63	977	
Viburnum trilobum	High Bush Cranberry	158	2,362	
Symphoricarpos albus	Snowberry		15	
Rhus typhina	Staghorn Sumac	15	1,027	
Juniperus communis	Juniper	7	344	
Sambucus pubens	Red Elderberry	21	7,858	
Sambucus canadensis	Black Elderberry	6	2,417	
Crataegus mollis	Hawthorn	134	1,382	
Prunus virginiana	Choke Cherry	7	62	
Prunus pennsylvanica	Pin Cherry	15	233	
Celtis occidentalis	Hackberry	18	76	

*Species used to improve wildlife habitat.

Twenty eight species must be maintained for production of special stock for parks, highways, and institutional planting (Table 2). An additional 26 are classed as wildlife species (Table 3). These are used to improve wildlife habitat and food supply in wildlife management. These two programs are represented by small quantities of seed though of a wide range in species. In total, 75 species are currently maintained in storage at Angus.

The species in storage do not fully represent the total seed for the provincial silvicultural program. Other species are collected and sown immediately because of problems in storage or because adequate supplies can be obtained annually without storing.

Black walnut used to be processed at Angus, including a rotting process for the husk and eventually dehusking. Results were just as satisfactory when nuts were collected, transported to St. William nursery and sown immediately. Germination proved to be as good or better than before. A similar situation existed for butternut, though only limited quantities are sown.

Storage temperature was found to be highly critical for red oak. Uniform conditions were found in a root cellar over winter. White oak cannot be stored at all. In fact germination may occur on the tree before acorns are collected. Again immediate sowing is recommended.

Maples can be stored in current frozen conditions. Normally, however, it is more convenient to collect soft maples and sow immediately in the spring. Hard maples are similarly handled in the fall. Ash species are also collected and sown in the fall. Annual cone crops are normal for these species.

Hickories cannot be stored under present conditions, but are seldom included in regeneration programs.

Basswood proves to be inconsistent and incomprehensible. Germination varies from year to year, from excellent to terrible! If collected early and sown immediately, sowing densities are difficult to maintain without removal of wings.

Most wildlife species can be stored safely overwinter and sown the following spring. Viburnum species are safely stored for several years.

Unlike other conifers, white cedar loses viability rapidly after the second year in storage.

For the species currently in storage, 2 to 3°C below freezing is proving to be adequate to maintain good viability for the necessary duration between crop years. In fact the principal seed storage problem faced in Ontario has been to get sufficient seed into storage to meet the rapidly increasing silvicultural program. This is especially so with the irregular periodicity of black spruce at a time when seed requirements for this species for direct seeding of some upland sites is rapidly expanding. Ontario lacks the rural population most countries rely on for cone collection especially during the critical period between seed maturation and dispersal. However, new mechanized technology is being developed. When the necessary quantities of seed do arrive at Angus the storage facilities of the Ontario Tree Seed Plant are ready to handle the foreseeable quantities for some years to come.

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AN OUTLOOK OF SEED STORAGE PROBLEMS OF SOME USEFUL INDIGENOUS TREES IN TANZANIA

By I.M. Shehaghilo
Silviculture Research Station
P.O. Box 95, Lushoto
TANZANIA

ABSTRACT

Problems connected with storage of indigenous tree seeds as experienced in the past are noted, and how these problems would affect future expansion of afforestation work if unsolved, are viewed. Future research work and prospects on the more perishable important indigenous tree seeds are indicated.

RÉSUMÉ

On souligne les problèmes recontrés jusqu'à maintenant concernant l'entreposage des semences d'arbres indigènes et on examine les effets que ces problèmes auront sur l'expansion des travaux de reboisement d'extension s'ils ne sont pas résolus. On traite également de recherches envisagées sur les semences périssables d'essences indigènes importantes, et des résultats visés.

INTRODUCTION

Valuable indigenous trees were once in abundance, but due to heavy commercial exploitation, and slow growth rate after natural regeneration, many species were reduced and thus cannot be utilized. The government's plans have been to afforestate areas by planting fast growing exotic timber trees to augment the timber requirements. Afforestation with indigenous species was given less attention. Due to the above mentioned trend, little attention was directed to seed problems. When the need to grow more indigenous trees both naturally and artificially was realised, it became evident that seed availability and peripheral needs had to be considered. One of the problems being realised is the storability of the indigenous seeds. Many of them lose viability quickly under normal storage conditions. There is a need to go into a thorough research to find the best methods of prolonging the viability period of perishable seeds. Work has started with some storage experiments which are still at their early stages. The aim of this paper is to give an overall view of the past and present positions and future plans with regards to seed storage problems of the more valuable indigenous trees the seeds of which lose their viability rapidly. Thirteen valuable indigenous species are at present regarded as needing high priority in solving their storage problems.

PROBLEMS OF STORAGE

The germination of many indigenous tree seeds appear to be low, and its storage period appears to be short in many cases; the reasons for this not being fully understood. Little work on storage of seed of these indigenous species has been done in the past, because these species were in abundance naturally, and afforestation programmes were concentrated on exotics which were faster growing. Commercial exploitation of valuable indigenous species were carried out without thinking of ways of afforestating more areas with the exploited species. In a few cases, however, natural regeneration has been encouraged.

The valuable species are diminished and some are in danger of extinction, resulting in some areas and species to be closed for exploitation; for example *Ocotea usambarensis* in a larger part of Shume-Magamba Forest Reserve of West Usambara and Kilimanjaro Forest reserves, ***Beilschmiedia kweo*** in East Usambara, ***Olea welwitschii*** in Meru area and many others. The government has stopped or restricted their commercial exploitation and encourages natural regeneration by seed, suckers or coppices. However, progress is slow and the distribution of the trees are uneven in spacing and age. To introduce the species elsewhere one would need seeds. To get seeds may be a problem, but the main problem occurs in their storage. Many species are known to be perishable and little research has been done to find ways and means of increasing longevity in the past because seeds were not needed in large afforestation programmes. This trend is now changing, so now seeds will be needed for such programmes.

The other thing to note is that many of the indigenous trees do not flower and fruit regularly, so when seeds are collected in good seed years, they need to be stored over a number of months or years to meet annual targets until next harvest. This has up to now not been possible for many of our valuable indigenous species.

Shehaghilo (1979) mentioned and arranged tree seeds (including exotics) into four categories according to their longevity period as studied at normal room conditions. Table 1 is a list of those useful indigenous species which pose a big problem in storage and which should be given priority in finding out the best environmental conditions for storing their seeds.

Table 1. List of useful seed species which are difficult to store.

Allanblackia stuhlmanii
Beilschmiedia kweo
Cephalosphaera usambarensis
Entandrophragma excelsum (Edeiningeri)
Hagenia abyssinica
Khaya nyasica
Maesopsis eminii
Olea welwitschii (O. capensis)
Trichilia roka (T. emetica)
Brachylaena hutchinsii
Chlorophora excelsa
Juniperus procera
Ocotea usambarensis

Some of the above listed seeds will only store for a month after collection, although a few can store for about a year. The longest storage period found so far for storing seeds of *Olea welwitschii* in Tanzania was for five months (Kimariyo 1973). Shehaghilo (1979) found *Trichilia roka* to lose viability within two months after collection. In Uganda, Synnot (1973) also found *Entandrophragma utile* seed with an initial germination capacity of 100% to have lost viability down to 5% in the sixth month.

Nearly all the species listed in Table 1 have seeds which fall to the ground soon after maturity and germinate, though at different intensities. Many of these seed species appear to be specific in moisture and temperature requirements, and these conditions are unknown at present. Once storage conditions for each species is known, storage facilities can be designed in accordance with these requirements.

FUTURE WORK

A review of the literature only shows very general storage information. The results given are very helpful but they do not provide sufficient storage practices. There are too many exceptions to the general rules. Experiments have just started with a couple of species and results are not yet completed. It is intended to design experiments so as to study in detail the best storage conditions for each species which could be practised for large afforestation programmes in the future. Phenological observations carried out in the past are inadequate; therefore detailed studies will be carried out during various stages of development, from flowering to seed maturation and seedfall. The length of time of the seed on the ground in the forest to the time it is picked, with all the environmental conditions it is subjected to, are factors which contribute to its storability. These are some of the study areas to be looked into.

CONCLUDING REMARKS

In many respects wood quality of the indigenous hardwoods is superior to those of the exotics so far introduced in Tanzania. Encouragement of natural regeneration within the natural distribution of the species in question has been going on, but this is not enough because of the many blank areas in the forest.

Because most of these indigenous species have been over-exploited, the present merchantable products are very low. To increase this for the future demands, afforestation programmes are necessary. These will depend very much on the availability of larger quantities of viable seeds at regular intervals in order to meet annual targets.

Some trees do not produce seeds annually and the viability period of many is short, from two months to nearly a year. Storage conditions to prolong the viability period have not been intensively studied in the past because it was not necessary then. Now the necessity is apparent, and so scientific efforts need to be focused on this important discipline of studying indigenous tree seeds, especially on storage conditions, otherwise expanded afforestation programmes may be hampered.

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TEMPERATURE AND MOISTURE CONTENT CHANGES IN *PINUS SILVESTRIS* SEED STORED IN A CONTAINER DURING FREEZING AND WARMING

Kenneth Sahlén
Urban Bergsten

The Swedish University of Agricultural Sciences
Dept. of Silviculture, Sec. of Reforestation
S-901 83 UMEÅ, Sweden.

ABSTRACT

The use of a technique involving thermocouples connected to a datalogger for temperature registration has made it possible to continuously measure temperature changes in a seed container without disturbing the seed mass. This paper describes an experiment employing this method whereby temperature changes and seed moisture content were determined during freezing and warming.

During freezing of a 28-liter container of seed (14 kg) from +22 to −16°C, 36 hours elapsed before the center of the seed mass obtained the ambient temperature. After moving the container to room temperature it also required 36 hours before the temperature was completely uniform within the whole seed mass. On the average, the seed moisture content for the whole container increased during the freezing period and decreased again during the warming period. No stratification in seed moisture content between different parts of the container was evident.

RÉSUMÉ

L'utilisation d'une technique qui enregistre la température grâce à des thermocouples reliés à un enregistreur de données a rendu possible la mesure continue des variations de température dans un contenant de graines sans déranger la masse de graines. La présente communication décrit une expérience au cours de laquelle on a utilisé cette technique pour déterminer les variations de la température et du taux d'humidité des graines pendant leur refroidissement et leur réchauffement.

Au cours du refroidissement de +22°C à -16°C d'un contenant de 28 litres de 14 kg de graines, 36 heures se sont écoulées avant que le centre de la masse de graines soit rendu à la température ambiante. Après que le contenant eut été placé dans un endroit à température normale, il s'est encore écoulé 36 heures avant que la température à l'intérieur de la masse de graines ne soit devenue uniforme. En général, le taux d'humidité des graines dans la masse entière a augmenté pendant le refroidissement et a diminué au cours du réchauffement. On n'a observé aucune stratification du taux d'humidité des graines dans les différentes parties du contenant.

INTRODUCTION

In connection with seed storage, sometimes seed containers are moved from a cold storage to room temperature, or from room temperature to a cold storage. The difference in temperature and humidity between these two environments can be very great. As there is a possibility that the seed will be influenced by these strong outer environmental changes, it is important to know how the environment changes among the seed in a container. This paper describes a preliminary experiment to investigate these problems.

MATERIALS AND METHODS

For this experiment, ***Pinus silvestris*** seed with a germinability of 45% was stored in a plastic container with a capacity of 28 liters and a wall-thickness of 2.5 mm. Through one side-wall, holes were drilled at different levels for inserting thermocouples and for sampling purposes (Fig. 1). The holes used for sampling (2 cm in diameter) were first covered by one layer of tape, then a cover of the same plastic as that of the container and finally an additional layer of tape, with the aim of creating a wall over the holes as similar as possible in both material and thickness to that of the original, undamaged container.

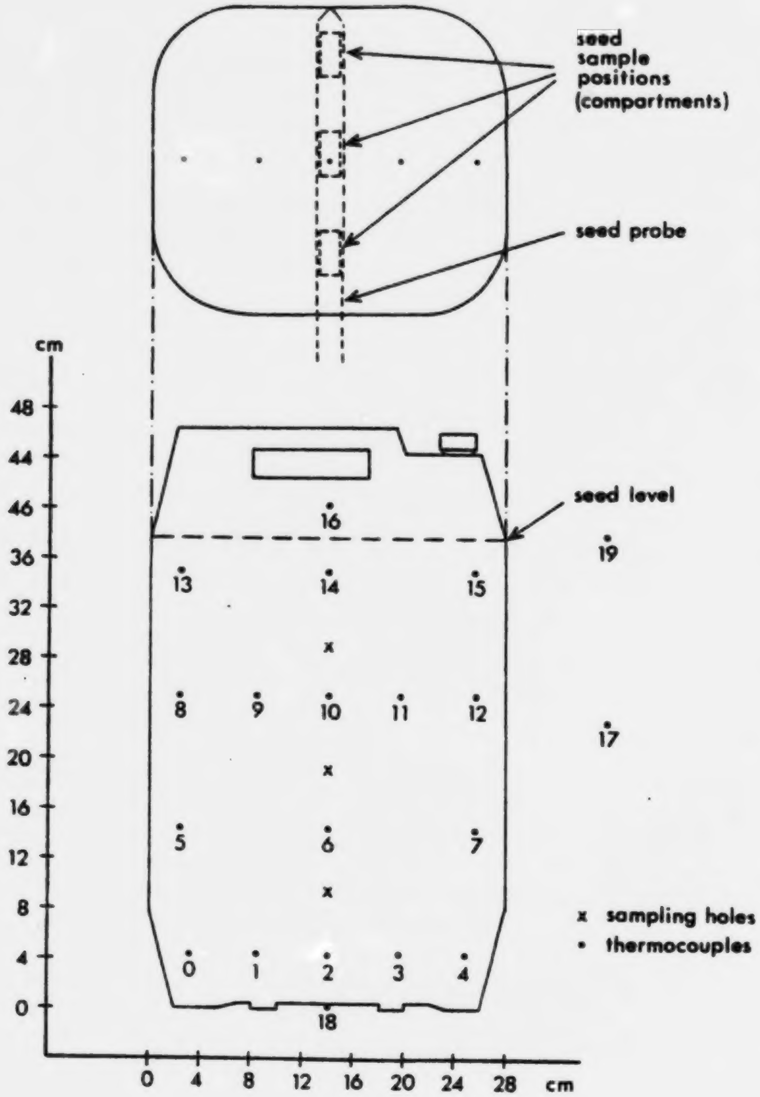
The thermocouples (TC) were of type Copper-Konstantan with a diameter of about 2.5 mm. The TC were connected to a datalogger Solatron 3430 for continuous temperature registration. The diameter of the connection wires was 0.25 mm. These wires were then passed through rubber corks. With this method, we obtained an accuracy in the temperature registration of ± 0.5°C.

Before starting the experiment, the seed was spread out on a table in the laboratory for 48 hours in order to achieve an even moisture content (measured to be 4.16%). The seed was poured into the container through the top hole up to the lowest level of TC. The TC were then put in position on the seed surface in the center of the container and at different distances from the side walls (cf. Fig. 1). The holes were sealed by rubber corks through which the TC wires were passed. Seed was then added up to the next level of TC, TC were inserted, and so on until the container was filled with 25 liters of seed. Between the container top and the seed surface was about 5 cm of airspace. The temperature in this gap was also measured, as well as the air temperature outside the container.

The container was subjected to a freezing and warming programme ranging from -16 to +22°C, with samples for determination of seed moisture content occurring at four different times according to the scheme in Fig. 2. During the freezing period, the container was placed on the floor in a freezing room and during the warming period, in the laboratory at room temperature.

Determination of seed moisture content

Before filling the container a sample was taken from the seed laying on the table for moisture content determination (S0). At the sampling occasions S1-S4 the following procedure was applied. The outer tape and the plastic piece were removed from the sampling hole. Then a seed probe with closed compartments was pressed through the inner tape and horizontally through the seed until it reached the opposite wall. The three compartments inside the probe were opened and filled with seed, and after that closed and the seed probe drawn out of the container.


seed sample positions (compartments)
seed probe
seed level
x sampling holes
• thermocouples
cm
48
44
46
36
32
28
24
20
16
12
8
4
0
0
4
8
12
16
20
24
28
cm
16
13
14
15
19
8
9
10
11
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17
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6
7
0
1
2
3
4
18

Figure 1. Size and design of the container used and position of thermocouples and sampling holes.

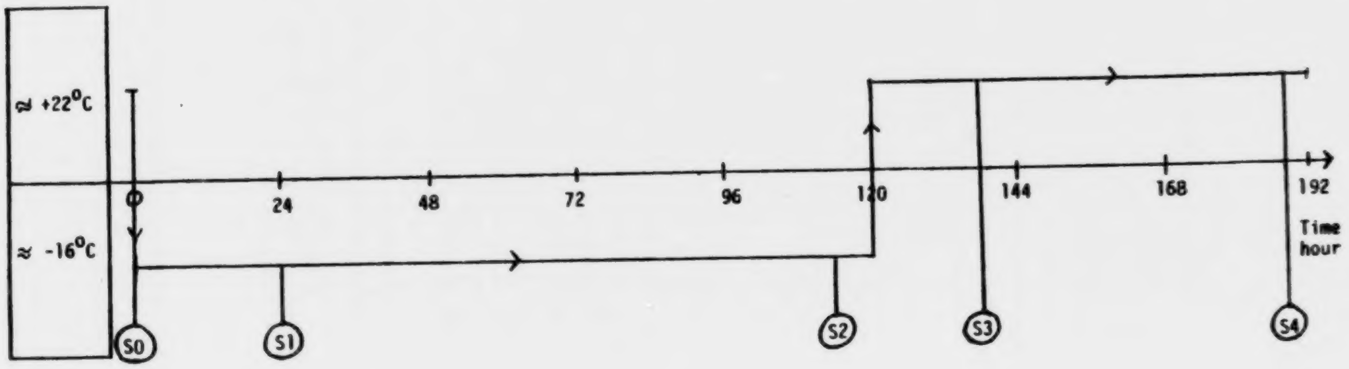

≈ +22°C
≈ -16°C
0
24
48
72
96
120
144
168
192
Time hour
S0
S1
S2
S3
S4

Figure 2. Freezing and warming programme and sampling times S0-S4 for determination of seed moisture content and relative humidity.

At the moment the seed probe left the container the sampling hole was sealed in the same way as before. At the three sampling times S2-S4, a sample was also taken from seed streaming out of the hole (i.e., seed laying just inside the container wall). The positions in the container of the samples (compartments) are shown in Fig. 1. Each compartment of the seed probe contained about 5 grams of seed.

This quantity was immediately divided into three subsamples. The seed moisture content was then determined by oven-drying at 105°C, and the value obtained for each position in the container is the mean of the three subsamples. Percentages are based on dry-weight.

At each sampling occasion the relative humidity was measured by a Vaisala Humicap humidity meter. Samples were taken in the air outside the container and in the air gap between the seed and the container top.

RESULTS

Temperature

During freezing, the temperature in the freezing room varied from -15.4°C to -18°C because of the thermostat regulation. The reduction in temperature was quicker in seed around the bottom of the container (TC 0, 4), and had reached the same value as outside the container after about 20 hours (Fig. 3). The time required for the other areas in the container to reach the outer temperature was 12 (TC 16), 30 (TC 1, 2, 3, 5, 7, 8, 12, 13, 15), 30 (TC 14) and 36 (TC 6, 9, 10, 11) hours (Table 1).

During warming the temperature in the container had reached 21°C (RT) after about 20 (TC 16), 26 (TC 0, 4), 26 (TC 13), 30 (TC 14), 32 (TC 1, 2, 3, 5, 7, 8, 12, 15) and 36 (TC 6, 9, 10, 11) hours (Fig. 4, Table 2).

Seed moisture content and relative humidity

Before starting the experiment, the seed moisture content (MC) was 4.16% and the relative humidity (RH) in the laboratory 21%. After 24 hours in the freezing room (S1), the MC had increased to 4.82%, and the RH inside the container decreased to 7% from 21%. 90 hours later (S2) the MC and RH were stabilized (Fig. 5).

After 18 hours of warming (S3), the MC and RH were 4.42% and 13% respectively. After 50 hours (S4), MC had decreased still more to 4.28% while the RH was unchanged. There was no tendency of stratification in the container at any of the sampling occasions.

DISCUSSION

As expected, the temperature first changes in the outer parts of the container, while it takes a considerably longer time before the whole container has reached outside temperature. The deviations in temperature of TCs 13 (Fig. 4) and 15 (Fig. 3) from TCs number 1, 2, 3, 5, 7, 8 and 12 are probably dependent on the influence both from above and from the side on these particular TCs.

Our hypothesis was that the gradient of temperature between the central and the outer parts of the container during the freezing and warming periods would

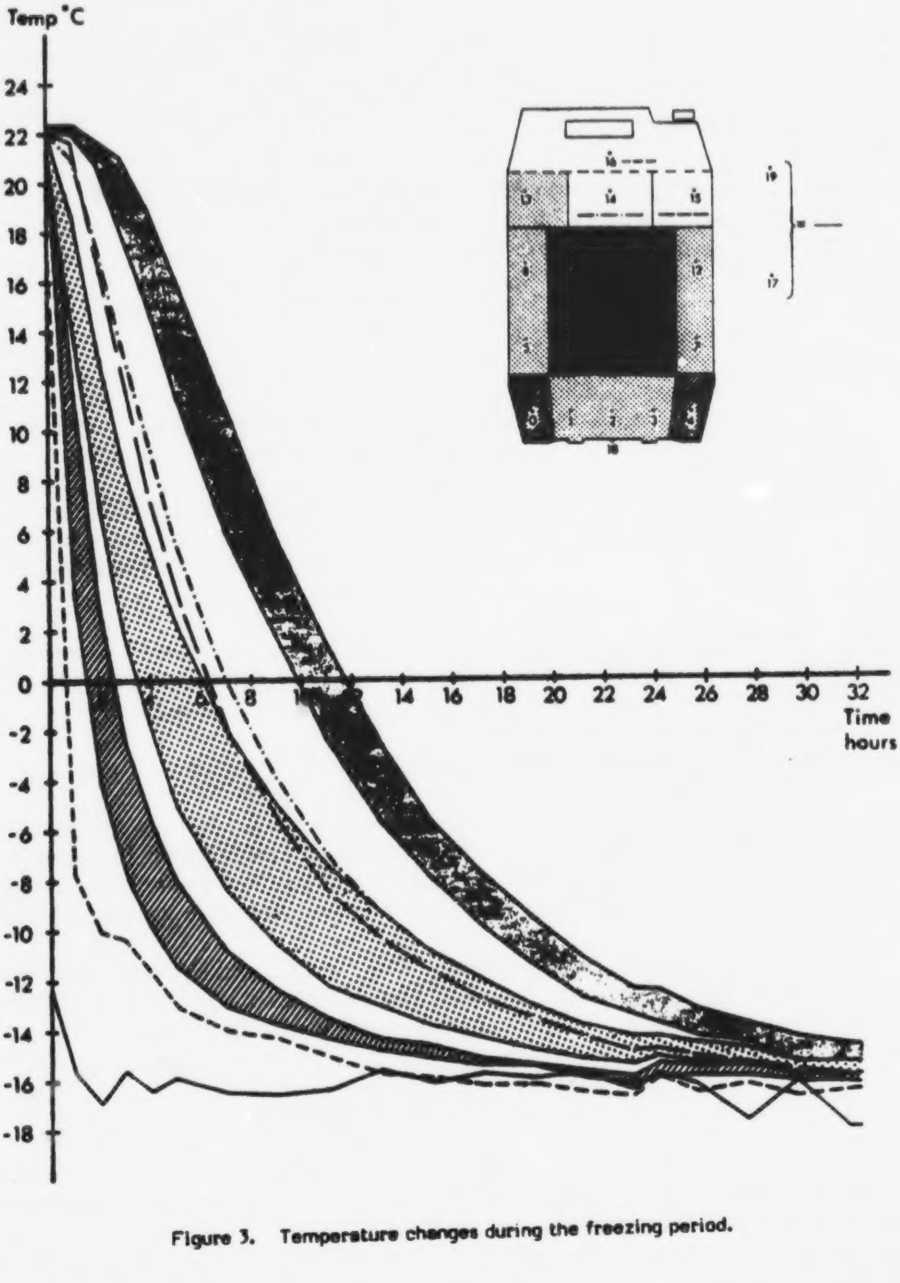


Figure 3. Temperature changes during the freezing period.

Time hours	Thermocouple nr.																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
0	21.2	21.5	21.7	22.0	21.5	21.5	22,2	22.0	21.5	22.2	22.4	22.4	21.7	21.7	22.2	22,2	18,7	-12.2	- 6.6	-12,2
1	3,7	15.3	14.0	18.5	11.8	15.5	22,2	18.3	17.3	22.0	22.4	22,2	18.7	15.8	21.0	21.2	-7,7	-15.7	-14,6	-15,7
2	-4,2	7.2	7.5	12.3	2.9	9.3	21,7	12,5	11.3	20,2	22.0	20.7	13,0	8.5	17,3	17,0	-10,0	-17,0	-15,7	-16.7
3	-7,7	1,6	3,2	7,2	-2,4	5.2	20.5	8,2	7.0	17,8	21.0	18,0	8.5	3,2	13,0	12,0	-10,3	-15,7	-15,1	-15,4
4	-9.8	-1,9	-0.1	3.4	-5,6	2.1	18,3	4,9	3,9	14,8	19.0	15,3	5,2	-0,4	9,3	7.5	-11,9	-16,5	-15,7	-16,2
5	-11,4	-5,0	-2,9	-0,1	-7,9	-0.9	15.5	2,1	1,1	11,5	16.3	12,0	2,4	-3,2	5,7	3,7	-13,0	-15,9	-15,7	-15,9
6	-12,2	-6,9	-5.0	-2,7	-9,5	-2,9	12,5	-0,4	-1.1	8,8	13,8	9 0	-0,1	-5,3	2.6	0,6	-13,5	-16,5	-15,9	-16,5
7	-13,0	-8,4	-6,6	-4,8	-10,8	-4,5	9,5	-2,4	-2,9	6,0	11,0	6,5	-1,9	-6,9	-0.1	-1,9	-14,0	-16,5	-15,9	-16,5
8	-13,2	-9,5	-8,2	-6,6	-11,6	-6,1	6,7	-4,0	-4,5	3,7	8,2	4,2	-3,5	-8,2	-2,2	-4,0	-14,3	-16,2	-15,9	-16,2
9	-13,8	-10,6	-9,2	-7,9	-12,4	-7,4	4,4	-5,6	-5.8	1,6	5,7	1,9	-5,0	-9,2	-4.0	-5,6	-14,3	-16,5	-16,2	-16,7
10	-14,0	-11,4	-10,0	-9,2	-13,0	-8,2	2,1	-6,6	-6,9	-0,6	3,4	-0,1	-6,3	-10,3	-5,6	-7,1	-14,8	-16,2	-15,9	-16,2
11	-14,3	-12,2	-10,8	-10,0	-13,5	-9,2	-0,4	-7,9	-7,9	-2,4	1,4	-1,9	-7,4	-11,1	-7,1	-8,4	-15,1	-16,5	-16,2	-16,2
12	-14,6	-12,7	-11,6	-10,8	-13,8	-10,0	-2,2	-8,7	-8,7	-4,0	-0,6	-3,5	-8,4	-11,6	-8,2	-9,2	-15,4	-16,5	-15,9	-16,2
16	-15,1	-14,0	-13,5	-13,0	-14,8	-12,4	-7,9	-11,6	-11,6	-9,0	-6,6	-8,4	-11,4	-13 2	-11,4	-12,2	-15,7	-15,4	-15,4	-15,4
20	-15,9	-15,1	-14,8	-14,6	-15,9	-14,3	-11,6	-13,8	-13,8	-12,2	-10,6	-11,6	-13,5	-14,8	-13,5	-14,0	-16,5	-17,3	-16,7	-17,0
24	-15,4	-15,1	-14,8	-14,8	-15,9	-14,6	-13,5	-14,3	-14,3	-13,8	-12,4	-13,2	-14,3	-15,1	-14,3	-14,8	-15,9	-15,9	-15,7	-15,7
36	-15,9	-15,9	-15,7	-15,7	-16,2	-15,7	-15,7	-15,7	-15,7	-15,9	-15,4	-15,4	-15,7	-15,9	-15,7	-15,9	-16,5	-16,2	-15,9	-16,2
48	-16,2	-16,2	-15,9	-16,2	-16,5	-16,2	-16,5	-16,2	-16,2	-16,5	-15,9	-15,9	-15,9	-16,2	-15,9	-16,2	-16,7	-16,7	-16,2	-16,5
72	-15,9	-15,9	-15,7	-15,9	-16,5	-16,2	-16,5	-15,9	-15,9	-16,5	-15,9	-15,9	-15,9	-16,2	-16,2	-16,2	-16,7	-15,7	-15,7	-15,7

Table 1. Temperatures in °C for the different thermocouples after freezing start.

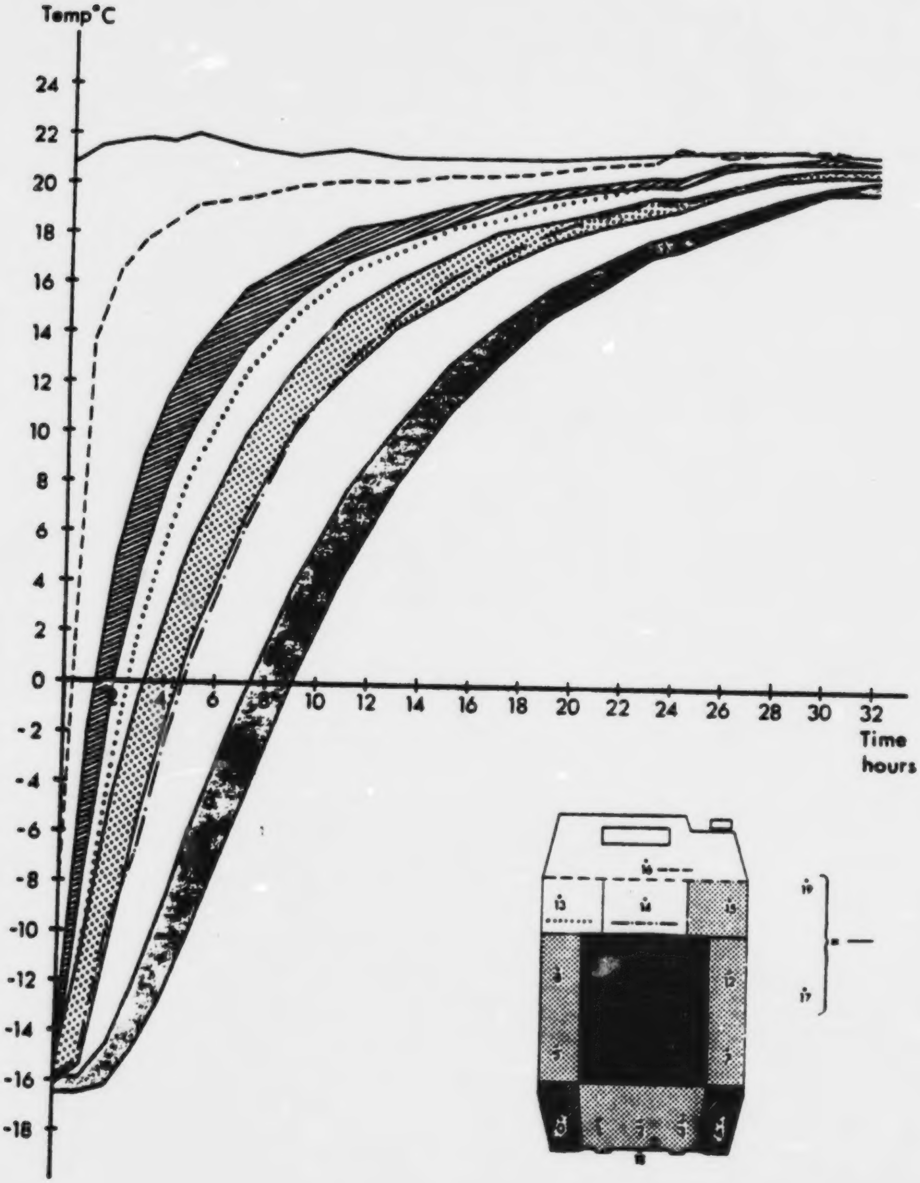
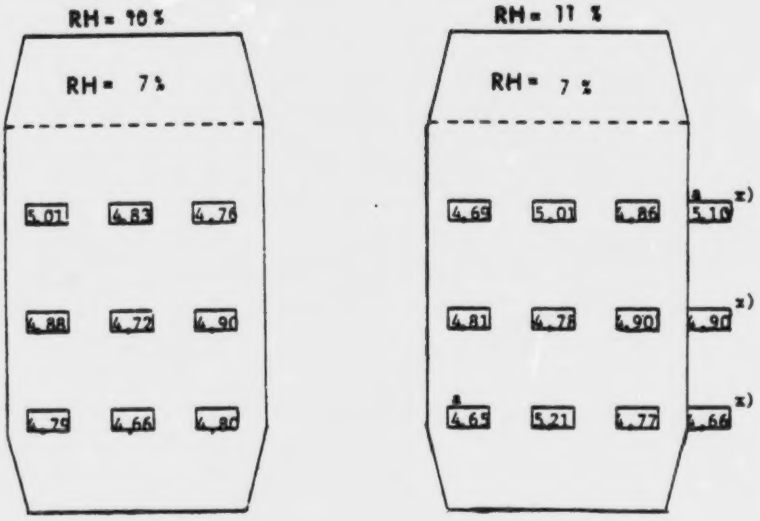


Figure 4. Temperature changes during the warming period.

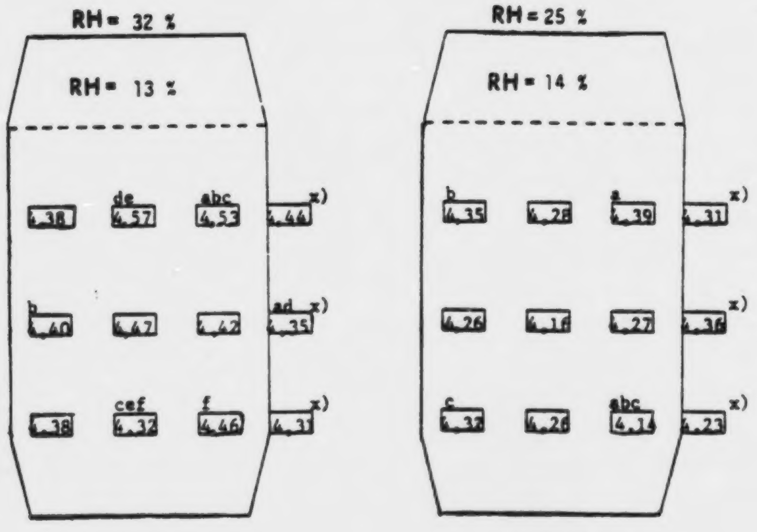
Time	Thermocouple nr.																			
hours	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
0	-15.9	-16.2	-15.9	-16.2	-16.2	-16.2	-16.5	-16.2	-15.9	-16.5	-15.9	-16.2	-15.9	-15.9	-15.9	-16.2	-8.4	21.0	5.5	20.7
1	-1.9	-11.6	-10.6	-13.5	-7.9	-10.8	-16.5	-13.0	-12.2	-16.5	-15.9	-15.9	-13.0	-10.8	-15.1	-15.4	13.5	21.5	3.9	21.5
2	5.2	-5.8	-6.6	-9.5	-0.4	-5.0	-16.2	-7.4	-6.1	-15.1	-15.7	-14.6	-7.4	-3.5	-11.1	-10.8	16.5	21.7	5.7	21.7
3	9.0	-1.1	-3.2	-5.6	4.7	-0.9	-14.8	-3.2	-1.9	-12.2	-14.6	-12.2	-2.9	2.1	-6.9	-5.8	17.8	22.0	7.7	21.7
4	11.5	2.6	-0.6	-1.9	8.0	2.4	-13.0	0.3	1.4	-9.5	-13.0	-9.5	0.3	5.7	-2.9	-1.4	18.5	21.7	9.5	21.7
5	13.3	5.7	2.4	1.6	10.3	4.9	-10.6	3.2	4.2	-6.3	-10.6	-6.3	3.2	8.5	0.6	2.6	19.2	22.0	11.3	22.0
6	14.8	8.2	5.2	4.4	12.3	7.2	-7.7	5.5	6.2	-3.5	-7.9	-3.5	5.7	11.0	3.7	5.7	19.7	22.0	12.8	22.0
7	15.8	10.0	7.2	6.5	13.5	8.8	-5.0	7.2	8.0	-0.9	-5.3	-1.1	7.2	12.3	6.0	8.0	19.5	21.5	13.8	21.5
8	16.5	11.8	9.3	8.5	14.5	10.3	-2.4	9.0	9.5	1.6	-2.4	1.9	9.0	13.8	8.5	10.0	19.7	21.5	15.0	21.7
9	17.0	12.8	10.8	10.3	15.5	11.3	0.3	10.3	10.8	3.9	-0.1	3.7	10.5	14.8	10.0	11.5	19.7	21.2	15.8	21.2
10	17.5	14.0	12.0	11.8	16.3	12.8	2.6	11.5	12.0	6.0	2.4	5.7	11.8	15.5	11.5	13.0	20.0	21.5	16.5	21.2
11	18.3	15.0	13.3	13.0	17.0	13.5	4.7	12.5	13.0	7.7	4.4	7.5	12.8	16.5	12.8	14.0	20.2	21.5	17.3	21.5
12	18.3	15.5	14.0	13.8	17.3	14.3	6.2	13.5	13.8	9.0	6.2	8.8	13.8	16.8	13.8	14.8	20.2	21.2	17.5	21.0
16	19.5	17.8	17.0	16.8	19.0	17.0	12.0	16.3	16.5	13.8	11.8	13.5	16.5	18.5	16.8	17.5	20.5	21.2	19.0	21.2
20	20.2	19.2	18.5	18.5	19.7	18.5	15.5	18.0	18.3	16.5	15.5	16.5	18.5	19.7	18.5	19.0	20.7	21.0	19.7	21.2
24	20.5	19.7	19.5	19.5	20.2	19.5	17.5	19.2	19.5	18.3	17.5	18.3	19.5	20.2	19.5	19.7	21.7	21.7	20.2	21.5
36	21.2	21.0	21.0	21.0	21.2	21.2	20.7	21.0	21.2	21.0	20.7	21.0	21.0	21.2	21.2	21.2	21.2	21.2	21.0	21.5
48	21.7	21.5	21.2	21.2	21.7	21.5	21.2	21.5	21.5	21.2	21.2	21.2	21.5	21.7	21.5	21.5	22.0	22.0	21.5	22.2
72	21.7	21.5	21.5	21.5	21.7	21.7	21.5	21.5	21.7	21.5	21.5	21.5	21.5	21.7	21.7	21.7	22.0	22.2	21.5	22.4

Table 2. Temperatures in °C for the different thermocouples after warming start.


RH = 90 %
RH = 7 %
5.01
4.83
4.76
4.88
4.72
4.90
4.79
4.66
4.80
RH = 11 %
RH = 7 %
a
5.10
x)
4.69
5.01
4.86
4.81
4.78
4.90
4.90
x)
a
4.65
5.21
4.77
4.66
x)

S 1 (24 hours)

S 2 (114 hours)


RH = 32 %
RH = 13 %
4.38
de
4.57
abc
4.53
4.44
x)
b
4.40
4.47
4.42
ad
4.35
x)
4.38
cef
4.32
f
4.46
4.31
x)
RH = 25 %
RH = 14 %
b
4.35
4.28
a
4.39
4.31
x)
4.26
4.18
4.27
4.38
x)
c
4.32
4.26
abc
4.14
4.23
x)

S 3 (136 hours)

S 4 (188 hours)

Figure 5. Seed moisture content in % for the sampling occasions S1-S4 (cf fig. 2) in the different parts of the container and relative humidity inside and outside the container.

Values with the same index are significant differed at 95 % level.
-"- without index are not -"- -"- 95 % -"-
x) Seed streaming out of the sampling hole.

also lead to a difference in seed moisture content between these parts. In this experiment we did not find such a tendency, perhaps because of the very low moisture content at the beginning of the experiment, and the timepoints chosen for sampling. It is possible that we would have found differences if we had done the first sampling after approximately 12 hours, and the third after 132 hours. As expected, we found that the moisture content increased during the freezing period and decreased during the warming period (see Figure 5). This is natural, as with a constant water content in the air, the RH increases up to saturation when the temperature decreases to -16°C and thus the seed can take up water from the air. In the same way, the seed loses some water to the air when the temperature again increases. The relative humidity inside the container should have gone up during the freezing period and down during the warming period. Our measurements gave the opposite results. The values of RH were also in some cases extremely low. Therefore, it is doubtful if the figures of RH are correct. The possibility of measurement errors is not excluded.

In further investigations, we will try to explain in more detail the interaction between temperature, relative humidity and seed moisture content in relation to seed storage.

THE STORABILITY OF CHIR PINE SEED AFFECTED BY RESIN TAPPING

By

R.C. Ghosh and R.C. Thapliyal
Forest Research Institute & Colleges,
New Forest, *Dehra Dun*,
INDIA - 248006

ABSTRACT

Chir pine seed collected from trees tapped for resin for 16 years at *Dehra Dun*, India, were tested for germination 3 and 4 years after harvest. The results indicate a faster rate of the loss of viability of seeds collected from tapped trees as compared to the seed collected from untapped trees in the same locality.

RÉSUMÉ

Des graines de pins Emodi *(Pinus roxburghii)* gemmés pendant 16 ans à Dehra Dun, en Inde, ont été soumises à des épreuves de germination trois et quatre années après leur récolte. Leur perte de viabilité a été plus rapide que celle des graines provenant d'arbres non gemmés du même endroit.

INTRODUCTION

Resin is a metabolic product characteristic of gymnosperms, particularly of the Coniferales. The physiological role of resin in these plants is as yet little understood, though its value as a raw material for various industrial products is well known. Pines are extensively tapped the world over for resin and in India Chir pine, *Pinus roxburghii* Sarg., syn. *P. longifolia* Roxb., trees above 30 cm d.b.h. are commercially tapped.

The effect of resin tapping on cone production and seed quality in Chir pine has been investigated by various workers. Champion and Pant (1932) investigated the effect of heavy and light tapping on cone size, seed germination and 'plant percentage'. Lohani and Kureel (1973) investigated the production of cone and germination of seed from tapped and untapped trees. This paper presents the results of a three year assessment of the effect of resin tapping on the storability of Chir pine seed.

MATERIAL AND METHODS

In the New Forest campus of the Forest Research Institute there are extensive plantations of Chir pine. Established in 1927, the plantations represent 11 seed sources derived from Western Himalayas including some areas now in Pakistan. Lohani and Kureel (1973) based their investigations on bulk samples of cone harvested from tapped and untapped trees of 9 out of 11 sources. At the time of cone harvest, the trees were 37 to 49 years old and were being tapped at weekly intervals in the past 16 years i.e., from 1956 to 1971. Seed of 8 sources was available from this collection and was germinated in April 1974 and 1975, i.e., 3 and 4 years after the date of harvest in April 1971. The seed was stored during this time in tightly sealed polythene bags at room temperature. The average temperature at New Forest during the period of the experiment varied from a minimum of 2.7° C in winters to a maximum of 37.2° C in summers. Laboratory germination tests were conducted with 4 replications of 50 seeds at 30° C using silica sand as the medium and illuminated (ca.3,000 lux) for 7 hours. The germination was observed for 21 days.

RESULTS

The cumulative mean germination, 73.2 and 77.5% of seed from tapped and untapped sources respectively, one year after collection showed no significant differences. Out of 9 origins, the differences in germination were significant only in seeds from Lansdowne and Rawalpindi origins and in both cases the germination of seed from tapped sources was lower than the seed from untapped sources (Lohani and Kureel 1973). Three years after collection the tapped sources declined in germination from 73.2 to 20.7% as compared to untapped sources where the decrease was from 77.5 to 37.4%. In other words, the germinability of seed from the tapped sources was 44.7% lesser than that of the seed from untapped sources. As indicated in Table I, the germination of seed from tapped sources was significantly lower as compared to untapped sources in all the origins. Four years after collection the germinability declined in all the sources. There was practically no germination in seeds from tapped sources. Among untapped sources Hazara, Chakrata and Lansdowne still had 4, 8 and 20% germination respectively.

DISCUSSION

Lohani and Kureel (1973) observed no significant differences in all but two origins, viz; Lansdowne & Rawalpindi in the germination percentage of seed from tapped and untapped sources one year after collection. Champion and Pant (1932) on the other hand, observed that germination as well as 'plant percent' (i.e., the field emergence and survival) is poorer for seed harvested from heavily tapped trees as compared to lightly tapped trees.

The deleterious effects of resin tapping on germination, vigour and viability of seed has been reported in ***Pinus halepensis*** (Crivellari 1956) and ***P. pinea*** (Philippis ***et al.*** 1966). Earlier reports indicate a steady decline in seed quality in ***Pinus sylvestris*** (Voronov 1962; Paramonov 1975) and a slight depression in seed vigour in ***P. nigra*** var. ***Calabrica*** (Crivellari 1958) with increasing periods of resin tapping. There are instances, however, where resin tapping had no effect, or had some ameliorating effect on the quality of the seed (Magini 1958; Velkov and Kaludin 1970; Paramonov 1970).

The effects of resin tapping on seed quality is an important aspect for detailed physiological investigations. The presence of resin pockets in some conifer seed, such as those of ***Cedrus*** and ***Abies*** species, is well known. The role of resin in these cases is not clearly understood. However, resin from fir seeds has been shown to have an inhibitory effect on the germination of spruce and pine seed. Gunia and Simak (1968) reported that the deposition of resin on the substratum from punctured resin pockets on the seed resulted in low germination capacity. They, however, ascribed the effect to the oxidation products of the resin which provided a good medium for mould growth, which in turn attacked the seed itself. Studies have been carried out on the metabolic rates of plant parts involved in the synthesis of the building blocks of oleo-resins. David and Bernard-Dagan (1957) found a slight increase in soluble sugars in the needles of ***Pinus pinaster*** as a result of resin tapping. In ***P. nigra***, resin exudation caused an increase in the photosynthetic rate concomitant with decreased synthesis of fatty acids and resin acids (Sul'gin 1967). Podol'skaya ***et al.*** (1973) on the other hand found higher levels of carbohydrates in the needles and young shoots of untapped ***Pinus sylvestris*** trees as compared with tapped ones. Further investigations are needed to elucidate the physiological processes in the tree as affected by prolonged tapping and the direct or indirect influence on seed quality.

Table 1. **Effect of different periods of storage on the germination percentage of Chir pine seed from tapped and untapped sources. (Percentages are based on 4 replications of 50 seeds).**

Origin of Seeds		Tapped			Untapped		
		Time of germination test from the date of cone harvest					
		1 yr$^+$	3 yrs.	4 yrs.	1 yr$^+$	3 yrs.	4 yrs.
1.	Hazara	75.0	4.6	nil	66.0	9.3	4.0
2.	Rawalpindi	65.0	x	x	80.0	x	x
3.	Kangra	79.0	5.0	nil	76.0	14.0	nil
4.	Chakrata	68.0	42.0	nil	81.0	58.0	8.0
5.	Dehradun	89.0	48.0	nil	82.0	64.0	nil
6.	Lansdowne	74.0	13.0	nil	89.0	41.0	20.0
7.	Nainital	72.0	12.0	nil	69.0	42.0	nil
8.	E. Almora	62.0	nil	nil	78.0	19.0	nil
9.	Darjeeling	75.0	41.0	nil	77.0	52.0	nil
	Mean	73.2	20.7	nil	77.5	37.4	10.6

+ Data of Lohani & Kureel (1973).

x Seed of this provenance were not available.

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MEASUREMENT OF SEED MOISTURE IN *CARYA OVATA* AND *JUGLANS NIGRA*

F.T. Bonner

Southern Forest Experiment Station
U.S. Forest Service
Starkville, Mississippi 39759 USA

ABSTRACT

Satisfactory determinations of nut moisture content can be made for practical use by oven drying method in the intervals of 9-17% for *Carya ovata* for 15 hours at 80°C and of 6-12% for *Juglans nigra* for 17 hours at 103°C. Samples of 5 nuts (both cotyledons and shells) should be cracked and mixed; the entire sample should be used for *C. ovata*, but only 25 g for *J. nigra*. Equilibrium moisture contents for *C. ovata* were 10.4 percent at 40 percent relative humidity and 15.1 percent at 95 percent relative humidity. Values for *J. nigra* at these same humidities were 11.1 and 20.4 percent moisture.

RÉSUMÉ

Il est possible de déterminer de façon satisfaisante, pour des motifs pratiques, le taux d'humidité des noix de *Carya ovata*, dans l'intervalle de 9 à 17%, en les faisant sécher au four pendant 15 h à 80°C et celui des noix de *Juglans nigra*, dans l'intervalle de 6 à 12%, en les faisant sécher pendant 17 h à 103°C. Pour ce faire, on doit casser et mélanger un échantillon de 5 noix (cotylédons et coquilles) et utiliser tout l'échantillon pour *C. ovata*, mais seulement 25 g pour *J. nigra*. Le taux d'humidité à l'équilibre chez *C. ovata* a été de 10,4%, à 40% d'humidité relative, et de 15,1%, à 95% d'humidité relative. Chez *J. nigra*, pour les mêmes humidités relatives, les taux d'humidité à l'équilibre ont été de 11,1 et de 20,4% respectivement.

INTRODUCTION

Seed moisture content is the most important factor in maintaining viability of seeds in storage, so we must know moisture content when seeds are prepared for storage and when lots are spot-checked during storage.

The testing rules of the International Seed Testing Association (ISTA 1976) prescribe moisture testing procedures for many, but not all, forest tree species.[1] Seeds of *Carya* and *Juglans*, which are not included in the Rules, are potentially troublesome because they are large and have high kernel fat content, 70.6 percent fat in *C. ovata* and 60.4 percent in *J. nigra* (Bonner 1971). The oven drying method of moisture determination can be risky for seeds with high fat content, and drying schedules should be standardized against more accurate methods before general use. If oven drying methods are inaccurate, then toluene distillation, or other laboratory methods, must be used. These methods are complex, time-consuming, and expensive. Their use may be justified in official testing, but the seed manager concerned with storage needs a simple yet fairly accurate method. This paper reports on the suitability of oven drying methods for moisture determinations in seeds of *C. ovata* and *J. nigra*. Other aspects of seed moisture as related to storage are also discussed.

MATERIAL AND METHODS

All seeds used in this work were collected in central Mississippi in 1973 *(C. ovata)* and 1976-78 *(J. nigra)*. Each lot was a combination of nuts from 3 to 5 trees. Husks were partially to completely removed from *J. nigra* nuts by water or hand scrubbing before testing. The nuts were floated in water, and empty nuts (floaters) were discarded.

I divided the lots into numerous sublots and adjusted their moisture contents to get a range of seed moisture across all sublots. In the adjustment, some sublots were soaked in water for various periods, and others were dried on laboratory benches. All sublots were then stored at 3° for 3 to 4 weeks to allow equilibration within each sublot container.

Four sublots from the middle of the seed moisture range were selected, and samples (including both cotyledon and shell) from each sublot were dried for 1, 2, 4, 6, 8, 12, 16, or 24 hours at 80° and 103°. Duplicate samples were drawn for moisture determinations by toluene distillation (ISTA 1976). Toluene data were compared with the oven data, and 1 or 2 potentially good oven schedules were selected for closer comparison with the toluene method (Bonner 1972).

In the final comparisons, 20 sublots were used in paired measurements of oven vs. toluene for *C. ovata* and 15 sublots for *J. nigra*. Accuracy of the oven methods as compared to toluene was tested by chi-square (Freese 1960). The accuracy goal was to be within 1.0 percent of the toluene value (95 percent level of confidence). Error limits were also calculated by the procedures of Rennie and Wiant (1978).

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[1] In keeping with ISTA rules, all moisture contents mentioned in this paper are based on percent of wet weight.

Other aspects of moisture determination on *J. nigra* nuts were tested:

(1) Intact vs. cracked nuts
(2) Complete vs. partial cleaning of nuts
(3) Best sample size--1, 2, 3, or 5 nuts.

At least 30 pairs of measurements were made for each. Significance of differences was tested by the "t" test.

Equilibrium moisture values were determined at 4°C with saturated salt solutions:

$ZnSO_4.7H_2O$--94.7% RH

$CaCl_2.6H_2O$--39.8% RH

These determinations were replicated at least 3 times.

All laboratory procedures were carried out as close to ISTA methods as possible.

RESULTS AND DISCUSSION

For *J. nigra*, differences between intact and cracked nuts or between complete and partial cleaning were not significant (Table 1). Most walnuts are de-husked before storage (Brinkman 1974), but they are not normally cleaned completely of husk material. The ability to get reliable moisture determinations on partially cleaned nuts should encourage seed managers to make these measurements.

Table 1. --Effect of sample condition on moisture determinations in *J. nigra* nuts.

Test	n	Means		"t" value	
		percent			
		Intact	Cracked		
Intact vs. cracked nuts	30	9.4	10.1	1.8219	(NS)
		Complete	Partial		
Complete vs. partial cleaning	35	12.5	12.2	2.0188	(NS)

Samples of 5 nuts gave the lowest standard deviation for *J. nigra*, although a sample size of 3 nuts was almost as good (Table 2). Because the bulk of 5 nuts was too large for easy drying in the ovens, the sample size was reduced to a manageable 25 g. Using cracked nuts also allows the sample to be drawn from 5 nuts instead of 1 or 2. In all subsequent measurements, 5 nuts were broken apart and mixed, and a 25 g sample was drawn. Five broken nuts were used for *C. ovata* also; their total weight was about 25 g.

Table 2. —Variation in moisture content of *J. nigra* nuts as related to number of nuts per sample. (15 replications per sample size).

No. of Nuts	Stand. Deviation
1	1.3918
2	0.6649
3	.4818
5	.3973

The oven time trials suggested the following drying schedules as potentially good:

C. ovata -- 15 hours at 80°C
J. nigra -- 17 hours at 103°C or 8 hours at 103°C

Direct comparisons with toluene distillation by paired measurements suggested that the drying times were satisfactory.

For *C. ovata* nuts, the oven method values averaged slightly less than the toluene values (Table 3) and were accurate to only within 2 percentage

Table 3. —Moisture content of *C. ovata* nuts as determined by toluene distillation and oven-drying for 15 hours at 80°C.

Sub-lot	Toluene dist.	Oven	Difference
		percent	
1	9.6	9.4	0.2
2	9.2	8.3	.9
3	8.4	8.9	-.5
4	15.0	12.9	2.1
5	9.5	8.7	0.8
6	9.7	9.4	.3
7	11.4	10.9	.5
8	9.7	9.3	.4
9	12.0	12.2	-.2
10	13.5	14.2	-.7
11	15.6	15.1	.5
12	15.8	14.9	.9
13	14.9	14.4	.5
14	16.3	15.4	.9
15	16.0	15.7	.3
16	17.2	15.7	1.5
17	15.8	15.4	0.4
18	16.1	15.9	.2
19	17.2	15.9	1.3
20	16.2	15.6	0.6
Mean	13.5	12.9	

X (2% diff.) $= \frac{13.89}{1.0413} = 13.34$ (NS)   Error limit $= \frac{(1.96)\quad(13.89)}{31.41}\ 1/2 = 1.3\%$

points of "true" (toluene) moisture. The calculated error limit for this method was 1.3 percent. This means that the oven determinations will fall within 1.3 percent of the toluene value unless a 1-in-20 chance has occurred.

For *J. nigra* nuts, 17 hours at 103° proved the best schedule (Table 4). The mean difference was 0.3 percent, and the calculated error limit was 0.8 percent, well within the goal of 1.0 percent. The shorter schedule, 8 hours at 103°, produced a mean difference of 0.5 percent and a calculated error limit of 1.0 percent.

Table 4. --Moisture content of *J. nigra* nuts as determined by toluene distillation and oven-drying for 17 hours at 103°C.

Sub-lot	Toluene dist.	Oven	Difference
		percent	
1	10.7	10.7	0
2	20.7	21.7	-1.0
3	20.3	21.0	-0.7
4	12.0	12.5	-.5
5	10.0	10.0	0
6	5.8	5.8	0
7	2.5	3.0	-.5
8	21.4	21.7	-.3
9	18.6	20.1	-1.5
10	16.2	16.4	-0.2
11	13.0	13.1	-.1
12	2.8	2.8	0
13	9.5	9.8	-.3
14	20.4	20.4	0
15	14.0	13.9	.1
Mean	13.2	13.5	

$$X \text{ (1\% diff.)} = \frac{4.48}{0.2603} = 17.2109 \text{ (NS)}$$

$$\text{Error limit} = \frac{(1.96)\quad(4.48)}{25.00}\ 1/2 = 0.8\%$$

Equilibrium moisture contents for the two species at 4°C were:

	RH (%)	
	40	95
C. ovata	10.4	15.1
J. nigra	11.1	20.4

High fat content of nut kernels is potentially troublesome, especially if the percentage of unsaturated fatty acids is high. The fatty acids in ***J. regia*** kernels have been reported as over 80 percent unsaturated (Hilditch 1956), but we do not have similar data for ***J. nigra*** or ***C. ovata.*** But even if percentages of unsaturated fatty acids in kernels of these nuts were high, the close agreement of oven data with toluene data (Tables 3 and 4) indicates a minimum problem. Moisture determinations to within 1 percent of true moisture should be close enough for this purpose.

Rapid moisture determinations, such as those done for small seeds in electric meters, are not generally available for the large nuts. Rapid estimates have been obtained for *C. ovata* nuts with microwave oven drying with an error limit of only 1.7 percent (Bonner and Turner 1980), but better accuracy is generally desirable. The 15-or 17-hour drying schedule is convenient, because samples put into the oven near the close of a work day are ready for weighing the following morning.

Once seeds are stored at the proper moisture level, occasional measurements should be taken to detect any undersirable changes. The equilibrium moisture contents help in understanding these relationships. *C. ovata* nuts store well at 5 to 10 percent moisture (Bonner 1976), and ***J. nigra*** nuts are reported to store well at moisture contents of 16 to 33 percent or more (Brinkman 1974). So, storage atmospheres at 3° to 5° must be at or below 40 percent relative humidity for *C. ovata,* while humidities of over 95 percent are needed for ***J. nigra.*** The seed manager has two choices for *C. ovata:* (1) maintain 40 percent or lower relative humidity in the cooler, or (2) adjust nut moisture to below 10 percent and store in moisture-proof containers. Airtight storage of seeds with high moisture contents is generally not desirable, so ***J. nigra*** nuts should be stored at 95 percent humidity in containers that allow gas exchange. Both species should be stored 1 or 2° above freezing.

Although other ***Carya*** and ***Juglans*** species were not included in these tests, these procedures should work for other species of the same genera.

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ACCELERATED AGING STUDIES OF SEEDS OF JACK PINE *(PINUS BANKSIANA* LAMB.) AND RED OAK *(QUERCUS RUBRA* L.)

J.A. Pitel

Canadian Forestry Service,
Petawawa National Forestry Institute
Chalk River, Ontario, K0J 1J0, Canada

ABSTRACT

Artificial aging, at 43°C with high humidity, of seeds of jack pine (*Pinus banksiana* Lamb.) and red oak (*Quercus rubra* L.) resulted in a progressive decrease in their germination together with a decrease in the fresh weight and length of the germinating seedlings. Various biochemical analyses were performed at various periods of aging. For jack pine, increasing periods of aging resulted in an increase in the electrical conductivity and amount of amino acids from the seed steep-water. The soluble amino acids of the seeds increased with aging, together with changes in their percentage distribution. Electrophoretic analyses revealed minor changes in the type and quantities on polyacrylamide gels of isozymes of several enzyme systems; of the histone proteins; of the nonhistone chromosomal proteins; and of the ribosomal proteins during aging. No detectable electrophoretic changes were observed for RNA (ribonucleic acid).

For oak, the highest levels of soluble amino acids occurred after 6 days of aging. Some minor quantitative and qualitative changes were observed for isozymes of 4 enzyme systems during aging.

The results of this preliminary study indicate that the technique of accelerated aging can be useful to provide further knowledge of the mechanisms associated with the deterioration of forest tree seeds in storage.

RÉSUMÉ

Le vieillissement artificiel, à 43°C et à humidité élevée, de graines de pin gris (*Pinus banksiana* Lamb.) et de chêne rouge (*Quercus rubra* L.) en réduit progressivement la germination de même que le poids frais et la longueur des graines en cours de germination. On a effectué diverses analyses biochimiques à diverses périodes de vieillissement. Chez le pin gris, la conductivité électrique et la quantité d'acides aminés provenant de l'eau de trempe des semences augmentent avec la période de vieillissement. La teneur des graines en acides aminés solubles a augmenté et le pourcentage relatif de ces composés a changé avec le vieillissement. L'électrophorèse a révélé que, pendant le vieillissement, il se produit sur les gels de polyacrilamide de légères modifications quant à la nature et à la quantité des isozymes de plusieurs systèmes enzymatiques, des protéines d'histone, des protéines chromosomiques non histones et des protéines ribosomiques, mais aucune quant l'ARN (acide ribonucléique).

Dans le case du chêne, les plus fortes teneurs en acides aminés solubles ont été observées après 6 jours de vieillissement. De légers changements quantitatifs et qualitatifs ont été remarqués dans quatre systèmes enzymatiques pendant le vieillissement.

Les résultats de cette étude préliminaire indiquent que la technique de vieillissement accéléré peut servir à approfondir nos connaissances sur les mécanismes associés à la dégradation des graines d'arbres forestiers stockées.

INTRODUCTION

The fundamental causes of deterioration and loss of germination of seeds in storage are still not well understood. In studies with agricultural seeds the decline in viability has been associated with the deterioration of cell membranes; the reduced activity of many enzymes; decreased respiration; chromosomal abnormalities; impairment of the DNA (deoxyribonucleic acid) - RNA (ribonucleic acid) templating mechanisms; reduced rate of protein synthesis; accumulation of toxic materials; and others (for reviews see Abdul-Baki and Anderson 1972; Anderson 1973; Harrington 1973; Roberts 1973; and Osborne 1977). However very little is known of the physiology and biochemistry of seed deterioration of tree seeds. An understanding of the mechanisms which control the rate of seed deterioration might provide clues for developing methods of slowing down senescence and thus extending the life of the seeds.

The main purpose of this preliminary study is to examine at the molecular level some of the mechanisms associated with seed deterioration of jack pine and red oak using the technique of accelerated aging (Delouche and Baskin 1973). In this method the major symptoms of natural aging are induced over a relatively short span by exposing the seeds to conditions of high temperature and humidity. Thus seeds with different degrees of loss of vigour and viability can be rapidly obtained and examined biochemically. Accelerated aging techniques can also be used to determine the relative storage potential of different seed lots and for evaluating seed vigour and plant performance (Delouche and Baskin 1973). In this study, seeds with various periods of aging were examined for germination, leachate composition, amino acid composition, and polyacrylamide gel electrophoresis of isozymes, structural proteins and RNA.

MATERIALS AND METHODS

Seed Samples and Method of Accelerated Aging

Red oak and jack pine seeds studied were freshly collected from Chalk River, Ontario (Latitude 46°00'N, Longitude 77°26'W, and elevation of 150 m). Viability of the red oak acorns was 98% by the Tetrazolium Test. Seeds were subjected to accelerated aging by incubation for periods up to 18 days in a covered water bath maintained at 43°C and relative humidity of approximately 100%. No free water came in contact with the seeds. Seeds were air-dried to their original weight before use. Before analyses seeds were surface-sterilized with 5% (w/v) calcium hypochlorite. Jack pine was tested for viability on each sampling date by testing 3 replicates of 100 seeds on moist cellulose paper in plastic trays at 20°C in continuous light (360 μ einsteins m^{-2} sec^{-1}) for 14 days. Protrusion of the radicle for 2–3 mm. was used as a germination criterion. Unaged and aged seeds of jack pine were measured for fresh weight and length of germinants after 14 days of germination using only seeds that had germinated. Significance of means were determined by Duncan's multiple range test (Steel and Torrie 1960).

Conductivity Tests

Three replicates each of 1 gm (approximately 250 seeds) of jack pine seeds were soaked in 50 ml. of deionized water and incubated for 24 h at 20°C. The electrical conductivity of the resulting steep-water was measured with a conductivity meter. Red oak was not tested.

Amino Acid Analyses

Seeds of jack pine and red oak, before and after aging, were extracted in duplicate with 70% ethanol (v/v) using a Vir Tis homogenizer. The mixture was filtered and the residue re-extracted twice with 70% ethanol (v/v). The combined ethanol fractions containing the soluble amino acids were dried and then dissolved in 0.1M Na citrate buffer at pH 2.2. The residue remaining after ethanol extraction was dried, weighed, and then hydrolyzed in an autoclave at 110°C for 18 h with 6N HCl to obtain most of the insoluble, or bound, amino acids. The liquid fraction containing the insoluble amino acids was first dried and then dissolved in 0.1M Na citrate buffer at pH 2.2. Determination of amino acids was performed on a Beckman, Spinco, model 120C analyzer by the method of Benson and Patterson (1965). Aliquots of 1 ml. of each of the soluble and insoluble fractions were applied to the column of the amino acid analyzer.

To examine the amino acids that leached from seeds of jack pine, duplicate samples of 3 g of unaged and 16-day-aged seeds were placed in 50 ml. of deionized water at 20°C for 24 h. The steep-water was then dried and dissolved in 0.1M Na citrate buffer at pH 2.2 and examined for amino acids on the amino acid analyzer.

Isozyme Analyses

Isozymes of jack pine and red oak were examined using isolated embryos. Tissues were macerated at 4°C by mortar and pestle with an extraction buffer as described by Feret (1971). Tissue homogenates were centrifuged for 30 min. at 10,000 RPM. The supernatant was collected and centrifuged at 30,000 RPM for 30 min. The extracts were analyzed the same day as their extraction on 7.5% polyacrylamide gels by the disc electrophoresis method (Davis 1964). Peroxidase was assayed with the gels placed in a solution containing 15 ml. of 1% hydrogen peroxide in distilled water and 15 ml. of 2% (w/v) benzidine dissolved in 20% acetic acid in distilled water. Isozymes of leucine aminopeptidase, esterase and glutamate-oxaloacetate transaminase were identified by the methods of Guries and Ledig (1978), Berry and Franke (1973), and Adams and Coutinho (1977) respectively. For each stage of aging two independent extractions were performed and for each extract and enzyme analyzed, six replicate gels were run. For each gel tube 0.5 mg. of protein was applied.

Analyses of Chromosomal Proteins, Ribosomal Proteins and RNA

Histones and nonhistone chromosomal proteins (NHCP) were extracted from jack pine seeds and characterized by electrophoresis on polyacrylamide gels using the methods described by Pitel and Durzan (1978). Ribosomal proteins from jack pine were extracted and characterized by electrophoresis (Pitel and Durzan 1977). RNA (ribonucleic acid) was extracted from jack pine seeds and characterized on 2.5% (w/v) gels as described by Durzan *et al.* (1972) and Pitel and Durzan (1975).

RESULTS AND DISCUSSION

The results show that various biochemical changes occurred in seeds of both jack pine and red oak during aging. For jack pine, increasing periods of aging resulted in a decrease in germination together with decreases in the fresh weight and length of germinating seedlings (Table 1). Although the germination in the first four days of treatment was essentially the same, the rate of germination decreased slightly for the 2 and 4 day treated seeds and this was reflected by a decreased fresh weight and seedling length as compared with the untreated seeds. Measurements of conductivity of the seed steep-water during aging showed that as germination decreased the electrical conductivity increased. There were high correlation coefficients between percentage germination and growth measurements as shown in Table 1. The increase in conductivity that occurred with aging is thought to be due to changes in the molecular structure of the membrane, whereby pores are formed as a result of oxidation of the lipid portion of the membrane (Koostra 1973). Cytological studies with the electron microscope have shown the presence of damaged membranes of aged seeds of Scots pine (*Pinus sylvestris* L.) (Simola 1974). Parrish and Leopold (1978) have suggested that one symptom of aging is the deterioration of the ability of the membrane to reform and function upon hydration.

Table 1. Effect of accelerated aging on germination, conductivity, fresh weight, and seedling length of jack pine seeds. Within the down columns, percentages not followed by the same letter are significantly different at the 5% level. Correlation coefficients were determined between percentage germination and measurements for each of conductivity, fresh weight and length.

DAYS AT 43°C	GERMINATION %	CONDUCTIVITY (µm hos)	MEAN FRESH WEIGHT (g.)	MEAN SEEDLING LENGTH (mm.)
0	97.0 a	30.1 a	2.49 a	88.7 a
2	96.0 a	31.4 a	2.52 a	79.9 b
4	95.3 a	30.7 a	1.95 b	77.6 b
6	84.0 b	38.5 b	1.87 b	65.0 c
8	64.6 c	42.4 c	1.38 c	49.8 d
10	56.0 d	60.9 d	0.86 d	47.1 d
12	28.3 e	83.3 e	0.42 e	41.8 e
14	12.3 f	88.0 f	0.27 f	38.2 e
16	3.0 g	132.8 g	0.05 g	18.9 f
18	0 h	151.5 h	-	-
"r"		-0.9522	+0.9717	+0.9498

Table 2. **Percentage distribution of soluble amino acids of jack pine seeds at various days of accelerated aging.**

AMINO ACID	DAYS OF ACCELERATED AGING 0	4	8	10	14	16
ASPARTIC ACID	6.65	7.21	4.15	4.07	2.54	2.73
THREONINE	1.77	3.88	4.02	4.71	4.95	5.35
SERINE	6.16	6.05	6.16	5.93	5.55	5.66
AMIDES	4.18	11.90	4.35	4.89	4.26	4.69
HOMOSERINE	0.10	0.09	0.14	0.08	0.07	0.06
PROLINE	7.12	12.32	12.42	12.13	10.36	9.35
GLUTAMIC ACID	11.99	5.04	4.02	5.12	3.86	5.06
CITRULLINE	0.20	0.12	0.18	0.04	0.08	0.13
GLYCINE	1.33	1.02	1.64	2.15	4.95	5.21
ALANINE	27.00	11.22	25.35	17.36	18.56	15.54
α - AMINOBUTYRATE	0.14	0.07	0.06	0.05	0.03	0.04
VALINE	4.06	7.94	7.19	8.57	7.03	7.66
METHIONINE	0.22	0.10	0.09	0.08	0.91	1.81
ISOLEUCINE	2.19	5.14	4.56	5.96	4.70	5.46
LEUCINE	3.06	7.81	7.08	9.01	7.46	8.90
TYROSINE	8.17	8.79	8.13	8.84	7.58	7.78
PHENYLALANINE	1.99	3.29	3.36	3.46	2.68	3.23
β - ALANINE	0.28	0.28	0.23	0.16	0.09	0.14
γ - AMINOBUTYRATE	4.16	1.00	1.47	1.63	2.51	4.17
ORNITHINE	1.13	0.32	0.18	0.66	0.57	0.32
LYSINE	0.26	0.38	0.41	0.52	1.47	0.96
HISTIDINE	0.57	0.30	0.28	0.27	1.08	1.19
ARGININE	7.27	5.76	4.53	4.29	8.73	4.55
TOTAL µg.N/g. fr. wt.	31.78	65.69	69.05	93.96	194.96	212.25
TOTAL µg./g. fr. wt.	222.06	475.53	503.43	704.10	1319.93	1543.22

The soluble amino acids of jack pine were characterized by high levels of proline, glutamic acid, alanine, tyrosine and arginine (Table 2). During aging, changes were observed in the percentage distribution of many of the amino acids. There was a general decrease associated with aspartic acid and glutamic acid while the percentage distribution of threonine, glycine and leucine increased. For alanine there was a sharp drop after 4 days of aging, followed by an increase after 8 days and then a gradual decrease with further aging. During aging the quantities of the soluble amino acids increased 7 times (Table 2). This suggests that the breakdown of proteins is occurring during aging of jack pine seeds. These observations are consistent with reports for agricultural seeds whereby aging was associated with the degradation of proteins to their constituent amino acids (Roberts 1973; Anderson 1973). The insoluble, or protein, amino acids of jack pine were characterized by high levels of aspartic acid, glutamic acid and arginine (Table 3). Only minor fluctuations were observed during aging with regards to both the percentage distribution and to the total quantities of the insoluble amino acids. Examination of the amino acids from the seed steep-water of jack pine seeds showed that 23 amino acids were present (Table 4). Highest levels were observed for aspartic acid, the amides, glutamic acid, glycine and alanine. After 16 days of aging the percentage distribution of many of the amino acids changed. For example, levels of the amides and glycine decreased sharply while levels of glutamic acid and lysine increased. Also the total amounts of amino acids that leached from the seeds increased 7.5 times during aging. These results suggest that membrane damage (e.g., Koostra 1973) had occurred during aging of jack pine seeds resulting in the greater leakage of amino acids. In studies with pollen of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) of different viabilities, Ching and Ching (1976) observed that the total amount of amino acids of the leachate from non-viable pollen was 10 times that of the highly viable one. In studies with aged Scots pine seeds, leakage of metabolites such as amino acids was used as an index of deterioration (Pehap 1973).

Table 3. Percentage distribution of insoluble (bound) amino acids of jack pine seeds at various stages of accelerated aging.

	DAYS OF ACCELERATED AGING					
AMINO ACID	0	4	8	10	14	16
ASPARTIC ACID	10.15	10.04	10.13	10.06	10.16	9.96
THREONINE	2.88	2.82	2.78	2.73	2.79	2.77
SERINE	6.31	6.31	6.40	6.85	6.82	6.64
PROLINE	7.39	7.02	7.14	7.04	6.94	7.08
GLUTAMIC ACID	21.80	21.39	21.18	21.72	21.61	22.07
GLYCINE	5.22	5.23	5.13	5.13	5.28	5.24
ALANINE	4.84	5.07	5.07	5.22	5.05	4.94
VALINE	4.55	4.57	4.61	4.45	4.26	4.39
METHIONINE	0.07	0.04	0.09	0.10	0.10	0.05
ISOLEUCINE	2.71	2.77	2.87	2.75	2.68	2.68
LEUCINE	7.00	7.23	7.26	7.23	7.05	6.89
TYROSINE	1.88	0.94	1.03	1.45	1.09	2.34
PHENYLALANINE	3.27	3.62	3.43	3.41	3.14	3.34
LYSINE	2.08	2.22	2.39	2.11	2.24	2.09
HISTIDINE	1.63	1.17	0.34	1.53	1.56	1.53
ARGININE	18.23	19.56	20.16	18.23	19.22	17.99
TOTAL mg.N/g. residue	27.76	28.84	27.12	26.44	29.12	30.10
TOTAL mg./g. residue	177.18	181.14	170.27	168.61	182.80	193.07

Table 4. **Percentage distribution of soluble amino acids of jack pine that leached into the water after soaking the seeds for 24 h.**

AMINO ACID	DAYS OF AGING 0	 16
ASPARTIC ACID	9.92	6.91
THREONINE	5.13	3.55
SERINE	7.33	4.36
AMIDES	13.25	4.17
HOMOSERINE	0.36	0.38
PROLINE	4.13	2.84
GLUTAMIC ACID	11.40	16.87
CITRULLINE	0.52	0.71
GLYCINE	10.84	3.02
ALANINE	11.63	8.52
α - AMINOBUTYRATE	0.22	0.48
VALINE	5.89	3.12
METHIONINE	0.84	0.90
ISOLEUCINE	3.66	5.54
LEUCINE	7.47	5.04
TYROSINE	2.84	4.54
PHENYLALANINE	2.33	2.70
β - ALANINE	0.38	0.90
γ - AMINOBUTYRATE	0.47	5.08
ORNITHINE	0.17	1.57
LYSINE	0.32	10.60
HISTIDINE	0.26	2.54
ARGININE	0.65	5.67
TOTAL µg. AA	38.99	293.78

The soluble amino acids of red oak acorns were examined after 0, 2, 4, 6, and 10 days of aging which resulted in viabilities (based on the Tetrazolium Test) of 100, 96, 92, 80, and 42% respectively. Fresh acorns contained high levels of proline, glutamic acid, alanine and arginine (Table 5). During aging the percentage distribution of proline decreased while that of γ - aminobutyric acid increased. Major fluctuations in the percentage distribution was observed for alanine and arginine. The quantities of total soluble amino acids were highest for acorns aged for 6 days. The insoluble, or protein, amino acids of red oak acorns were characterized by high levels of aspartic acid, glutamic acid, leucine and arginine (Table 6). As observed for jack pine, only minor fluctuations were present for both the percentage distribution and the total insoluble amino acids of red oak acorns during aging.

Table 5. **Percentage distribution of soluble amino acids of red oak acorns at various days of accelerated aging.**

AMINO ACID	DAYS AT 43°C 0	2	4	6	10
ASPARTIC ACID	2.28	2.50	1.34	2.87	0.26
THREONINE	1.70	1.56	2.36	2.28	2.54
SERINE	1.67	2.95	3.50	4.02	2.19
AMIDES	5.25	3.81	4.51	3.89	1.61
HOMOSERINE	0.19	0.08	0.10	0.05	0.03
PROLINE	14.00	11.30	11.10	11.05	3.11
GLUTAMIC ACID	37.61	28.96	34.35	32.54	31.11
CITRULLINE	0.90	0.36	0.36	0.27	0.46
GLYCINE	0.14	0.63	0.44	0.30	0.58
ALANINE	9.10	22.68	9.46	11.80	18.93
α-AMINOBUTYRATE	0.07	0.08	0.07	0.11	0.05
VALINE	2.51	3.86	3.37	3.32	3.93
METHIONINE	0.04	0.03	0.02	0.03	0.05
ISOLEUCINE	2.03	2.92	2.37	2.01	2.94
LEUCINE	1.52	2.66	0.99	0.78	2.63
TYROSINE	1.67	3.72	3.33	3.49	5.15
PHENYLALANINE	1.80	1.82	1.27	1.21	1.70
β-ALANINE	0.08	0.08	0.04	0.05	0.14
γ-AMINOBUTYRATE	1.99	3.56	1.14	3.85	12.00
ORNITHINE	1.17	0.70	1.18	1.28	0.49
LYSINE	0.41	0.76	1.67	1.93	1.58
HISTIDINE	0.32	0.21	0.75	0.56	0.32
ARGININE	13.56	4.79	16.28	12.30	8.18
TOTAL µg.N/gm. fr. wt.	220.28	291.41	253.26	326.43	207.88
TOTAL µg./gm. fr. wt.	1,506.38	2,183.63	1,656.10	2,238.64	1,502.54

Examination of the isozymes of four enzyme systems of jack pine during aging showed some minor changes in the types and quantities of bands (Fig. 1). For example, untreated seeds contain only one band of glutamate dehydrogenase whereas after aging several additional minor bands were present. However the isozymes of leucine aminopeptidase and peroxidase were essentially unchanged after aging. Some minor quantitative and qualitative changes were also observed with isozymes of four enzyme systems of oak embryos during aging (Fig. 2). For example, the aging treatment resulted in the production of several additional minor bands of glutamate dehydrogenase, leucine aminopeptidase and peroxidase. Loss of viability of seeds has been generally associated with a decrease in the activity of most enzymes except for the hydrolytic enzymes such as proteases and phosphatases which tend to increase with aging (e.g., Harrington 1973; Roberts 1973). In this study the production of minor bands with aging may be the result of protein denaturation. Harrington (1973) has suggested that protein denaturation, either by cross-linking in an individual protein molecule or by protein polymerization, is one of the major causes of aging.

Table 6. Percentage distribution of insoluble (bound) amino acids of red oak acorns at various days of accelerated aging.

AMINO ACID	DAYS AT 43°C				
	0	2	4	6	10
ASPARTIC ACID	12.09	12.31	13.20	12.74	13.15
THREONINE	3.66	3.79	3.99	3.62	4.38
SERINE	4.70	4.65	5.05	5.06	4.99
PROLINE	5.61	4.16	5.13	5.74	5.24
GLUTAMIC ACID	16.76	18.51	13.61	17.01	18.41
GLYCINE	5.48	5.21	5.55	5.44	5.38
ALANINE	5.12	5.61	5.85	5.82	5.87
VALINE	5.19	4.42	5.33	5.34	5.61
METHIONINE	0.17	0.12	0.17	0.17	0.13
ISOLEUCINE	3.77	4.19	4.30	4.22	4.16
LEUCINE	8.17	8.75	8.60	8.77	8.67
TYROSINE	3.62	3.47	3.31	3.59	3.36
PHENYLALANINE	3.88	3.89	3.71	4.25	4.03
LYSINE	7.14	7.15	5.86	6.33	5.73
HISTIDINE	4.40	3.43	2.50	2.71	2.28
ARGININE	10.23	10.34	8.83	9.20	8.60
TOTAL mg.N/gm. residue	6.67	7.50	8.06	7.78	7.80
TOTAL mg./gm. residue	45.07	51.25	57.08	54.46	55.67

The proteins of the chromosomes (the histones and the nonhistone chromosomal proteins) were next examined to determine if any structural changes occurred during loss of seed viability of jack pine seeds. The histones are involved in the nonspecific repression of DNA - dependent RNA synthesis, as well as in the structural organization of the chromatin. They display limited heterogeneity and general lack of tissue and species specificity. By contrast, the nonhistone chromosomal proteins (NHCP) are heterogeneous, display significant tissue and species specificity and exhibit specific changes in synthesis and phosphorylation associated with gene activity (Pitel 1977). Five main histones were detected in both untreated and aged seeds (Fig. 3a). Although no qualitative changes were observed, there was however a decrease in the percentage distribution of the very-lysine-rich F1 histone with loss of viability (Table 7). Observations for the NHCP show some minor qualitative and quantitative changes with loss of seed viability (Fig. 3b). These results suggest histone denaturation. Also, evidence that DNA becomes fragmented during aging (e.g., Osborne 1977) suggests that the DNA template for transcription of RNA is being seriously impaired. Cytological studies by Roberts (1972) has shown that chromosomal aberrations are commonly found in old seeds. Structural changes of the DNA and chromosomal proteins would be the main cause of such aberrations.

Table 7. **Percentage distribution of histone fractions of jack pine seeds after various periods at 43°C.**

	DAYS AT 43°C			
	0	4	8	16
F1	22.5	22.1	18.9	17.8
F2b; F2a2; F3	57.0	58.2	59.1	59.8
F2a1	20.4	19.7	21.9	22.4

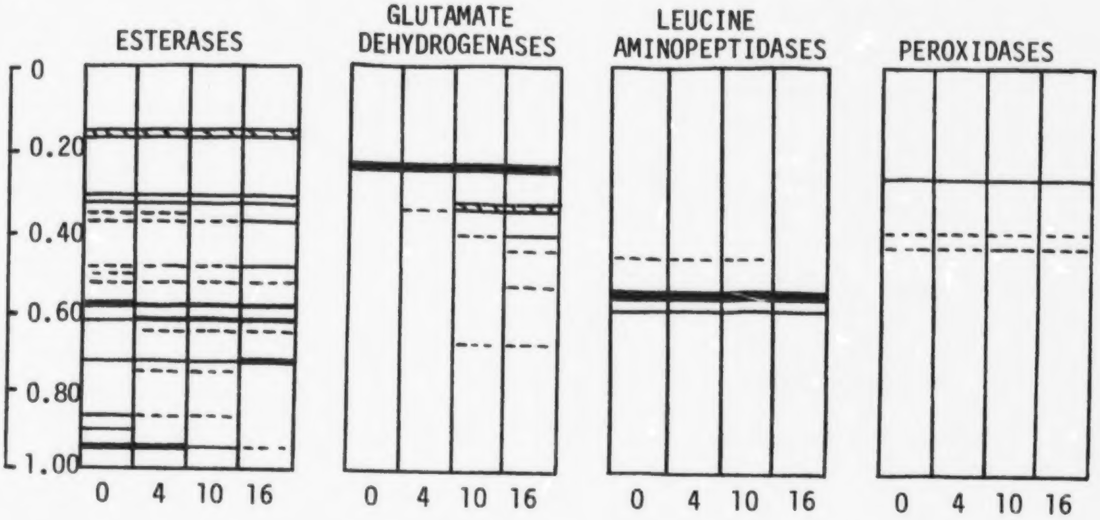
The structural integrity on polyacrylamide gels of the ribosomal proteins and RNA during aging of jack pine was examined. Total RNA was characterized by r (ribosomal)-RNA and t (transfer)-RNA (Fig. 4a). Although some evidence for other species shows that high molecular weight r-RNA is fragmented in non-viable seeds (e.g., Osborne 1977) these results did not demonstrate this. Degradation might occur during imbibition of the aged seeds, in which situation the nucleases would become more active (Osborne 1977). Although the RNA of aged jack pine seeds remained intact, this does not suggest that their synthesis is not impaired (Abdul-Baki and Chandra 1977). Loss of integrity of long-lived m (messenger)-RNA has also been shown to occur in aged seeds and may be the cause of reduced protein synthesis for these seeds upon hydration (Osborne 1977; Abdul-Baki and Chandra 1977). Another factor resulting in low protein synthesis may be the inability of the ribosomes of aged seeds to dissociate (App 1977). In the present study, examination of the basic ribosomal proteins of jack pine during loss of seed viability showed the presence of minor qualitative and quantitative changes (Fig. 4b).

CONCLUSION

The results indicate that loss of viability of red oak and jack pine seed is associated with various biochemical changes such as an increased leakage of membranes, catabolism of storage reserves, changes in the activity and structure of isozymes and possible denaturation of the chromosomal proteins. More studies however, are clearly needed to further define the mechanisms of aging with these species. One possibility would be to examine the phosphorylation of the chromosomal proteins of aged and imbibed seeds as this has been shown to be strongly associated with the rate and degree of growth and germination (Pitel 1977, Pitel and Durzan 1980). Studies are also planned to examine various constitutive and metabolic changes during "natural" aging of the seeds in cold storage.

ACKNOWLEDGEMENTS

Appreciation is expressed to Mr. Garry Scheer for operation of the Amino Acid Analyzer.


ESTERASES
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PEROXIDASES
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0 4 10 16

Figure 1. Distribution on 7.5% (w/v) polyacrylamide gels of isozymes of 4 enzyme systems of jack pine after various periods of seed aging.

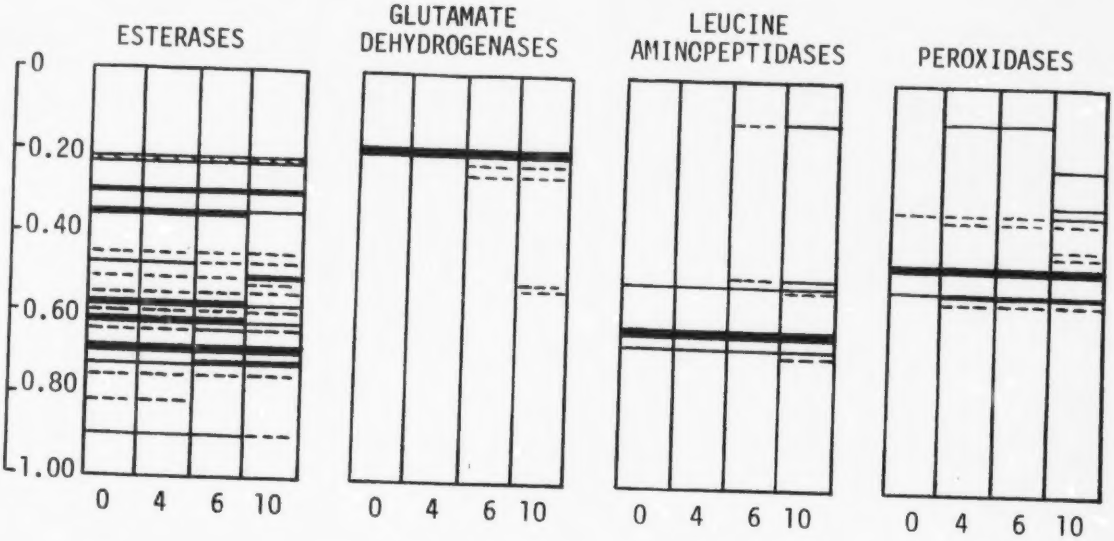
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Figure 2. Distribution on 7.5% (w/v) polyacrylamide gels of isozymes of 4 enzyme systems of red oak acorns after various periods of seed aging.

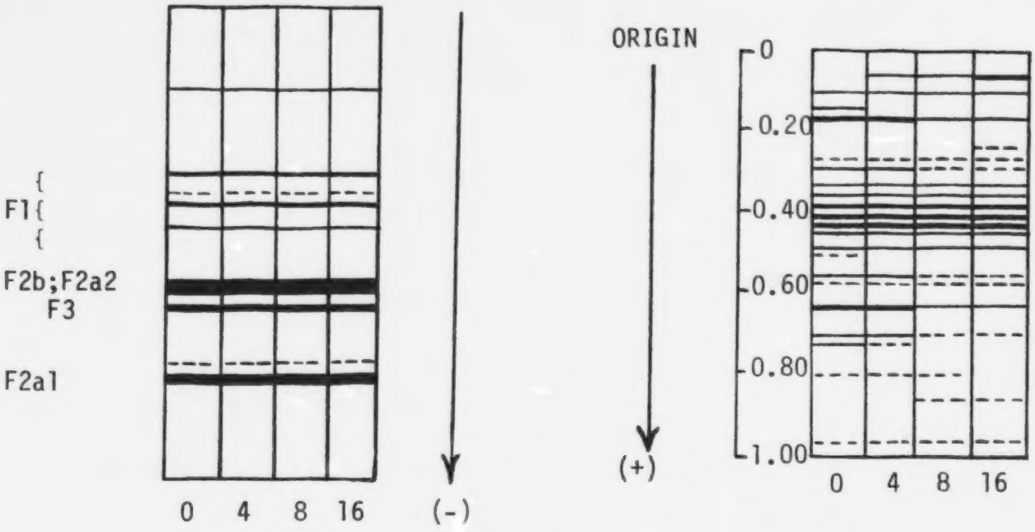
F1
F2b;F2a2
F3
F2a1
0
4
8
16
(-)
ORIGIN
(+)
0
0.20
0.40
0.60
0.80
1.00
0
4
8
16

Figure 3. Distribution on 7.5% (w/v) polyacrylamide gels of (a) histone proteins of jack pine after various periods of seed aging. In (b) the nonhistone chromosomal proteins of jack pine are examined.

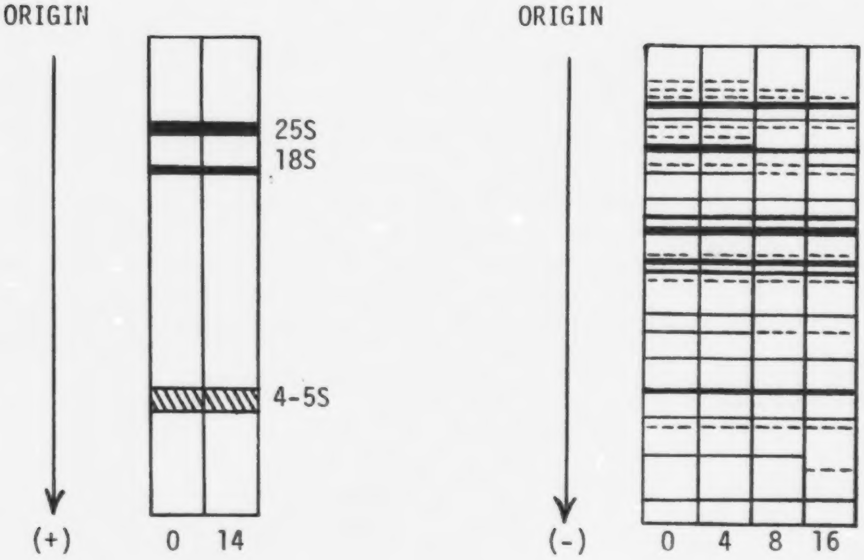
ORIGIN
25S
18S
4-5S
(+)
0
14
ORIGIN
(-)
0
4
8
16

Figure 4. In (a) total RNA of jack pine is examined with 2.5% (w/v) polyacrylamide gels. In (b) the basic ribosomal proteins of jack pine are examined on 7.5% (w/v) polyacrylamide gels after various periods of seed aging.

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SUMMARY REPORT

Technical Session 1: General problems and approaches to tree seed storage.

Chairperson: B. Suszka
Co-chairperson: C. Leadem

To store seeds of woody plants over periods longer than those possible in natural circumstances some basic conditions should be fulfilled. It is necessary to place sealed or open containers with seeds in controlled low-temperature conditions, which are provided by refrigerated chambers. These conditions together with the permissible moisture content should be known, especially for seeds losing viability easily. This knowledge can be attained only by well planned investigations, taking into account the biological possibilities of the studied seed material. When seeds are stored in sealed containers, it is important to know whether the temperature and moisture content of seeds does not undergo changes and deviations, which could be detrimental to the viability of seeds. Cones of some coniferous species are collected after a period of resin-tapping; the consequences of this procedure should also be well known. Seeds of some species are big, and best methods of estimating their moisture content, so important for storage, should also be found. The quality of the stored seeds decreases during storage because of natural ageing of their living tissues; therefore the process of ageing in various conditions should also be studied to find methods assuring a decrease of the intensity and rate of this process.

All these problems were the object of 6 papers presented by authors from Canada, Tanzania, Sweden, India and U.S.A. during Technical Session 1.

D. Altmann and L. Lafleur, Pine Ridge Forest Nursery, Smoky Lake, Alberta, Canada, have described underground seed cold storage facilities for 60,480 kg of spruce and pine seed which has been in operation for two years. The location of storage compartments, 2 m underground, is from the economical point of view the best solution in a climate where temperature can vary between -50° and +35°C during the year. Maintaining a relatively low temperature in the handling room at 4°C, will protect the frozen seed material from water condensation on their surface when the storage containers are opened. In the freezers where the seed is stored, temperature is held at a constant level of -18°C. Seeds are stored in heat-sealed plastic bags containing 10 kg and 20 kg capacity cardboard boxes. The storage facilities can store sufficient quantities of seeds for more than 10 years supply for direct seeding and nursery stock production requirement program of the Alberta Forest Service.

I.M. Shehaghilo, Silviculture Research Station, Lushoto, Tanzania, presented a situation in a tropical country, where it is difficult to collect the seeds in sufficient amount because valuable tree species are in danger of extinction. Seeds of many species lose viability quickly, but not much research has been done so far to find ways of extending the periods of their high viability and germinability. Storage of such seeds over some months or years is a necessity because of the irregular flowering and fruiting. The author listed 12 deciduous and 1 conifer seed species, which are very valuable but difficult to store. Data are available only for some of them and the possible longest storage periods found so far vary from several months to one year, depending on the species. Storage facilities for seed of these species could be constructed only when the moisture and temperature requirements of the seeds are known. If already started phenological observations and the studies on storage conditions are not intensified - the expanded reforestation and afforestation programmes may fail.

K. Sahlén and U. Bergsten, Swedish University of Agricultural Sciences, Umea, Sweden, have studied temperature and seed moisture changes of Scots pine in a seed container during freezing at a low moisture content from 22° to -16°C, and rewarming it again to room temperature. It was found that during freezing, temperature decreased slowest in the centre of the container and 36 hours were needed to bring it to the level of -16°C. After placing the container to room temperature it took also 36 hours until temperature became completely equilibrium in the whole seed mass. The seed moisture content increased during freezing and decreased during the thawing period. No stratification in seed moisture content between different parts of the container was evident.

R.C. Ghosh and R.C. Thapliyal, Forest Research Institute and Colleges, New Forest, Dehra Dun, India, have presented results of their studies on changes in germinative capacity of *Pinus roxburghii* Sarg. seed collected from trees tapped for resin for 16 years. The seed was stored for 3 and 4 years in sealed plastic bags at room temperature, in conditions of India. It was found that after storage over 1 year the mean germinability of seeds was still high and differences between seeds from trees tapped and non-tapped were insignificant. Seeds from tapped trees lost germinability totally after storage longer than 3 years, but seed from non-tapped trees germinated with 0 to 20% after 4 years of storage, depending on provenance. After 3 years of storage seeds from non-tapped trees germinated an average of 16.7% better than those from tapped trees.

F.T. Bonner, U.S. Forest Service, Southern Forest Experiment Station, Starkville, Mississippi, has compared two methods of measurement of moisture content of *Carya* and *Juglans* seeds: the toluene distillation method and the oven drying at 80°C and at 103°C. He found that it is possible to estimate moisture content of such seeds by the oven method in spite of their high fat content. However, for each species, appropriate temperature and duration of drying had to be found separately, and the drying schedules had to be standardized against the more accurate distillation method.

J.A. Pitel, Canad. Forestry Service, Petawawa National Forestry Institute, Chalk River, Ontario, has studied biochemical changes in *Quercus rubra* L. and *Pinus banksiana* Lamb. seeds during artificial ageing at 43°C with high humidity. The aim of this study was to find whether the technique of accelerated ageing can be useful for quick assessment of seed storability and for studying the mechanisms associated with the deterioration of forest seeds in storage.

It was found that decrease of germinability of seeds and of fresh weight and length of seedlings of jack pine was accompanied by an increase of electrical conductivity and of amino acids from the leachate. The soluble amino acids of the seeds increased with ageing and their percentage distribution was changed. Type and quantities of isoenzymes of several enzyme systems changed insignificantly as well as the histone proteins, the nonhistone chromosomal proteins and the ribosomal proteins. No detectable changes were observed for RNA. For oak the highest levels of soluble amino acids occurred after 6 days of ageing. Some minor quantitative and qualitative changes were observed for isoenzymes of 4 enzyme systems during ageing. All these data indicate that loss of viability of tree seed is associated with an increased leakage of membranes, catabolism of storage reserves, changes in the activity and structure of isoenzymes and possible denaturation of the chromosomal proteins. The studies should be continued and constitutive, and metabolic changes during "natural" and "artificial" ageing should be compared.

TECHNICAL SESSION 2: THE EFFECTS OF CONE STORAGE CONDITIONS ON SEED

Chairperson: D.G.W. Edwards
Co-chairperson: Y. Lamontagne

SEED VIABILITY OF *ABIES, PICEA, AND TSUGA* AFTER STORAGE IN THE CONES

C. Leadem
Research Branch, British Columbia Ministry of Forests
Victoria, British Columbia, Canada

ABSTRACT

Cones of *Abies amabilis, Picea glauca, P. sitchensis, P. engelmannii,* and *Tsuga heterophylla* were placed in cone sheds or in refrigerated storage (2°C) for various periods up to six months. Cone samples were withdrawn monthly and extracted seeds were germinated to determine seed viability. At the end of six months, no differences in seed quality were found between shed and refrigerated storage. There were some instances of reduced viability after storage, but these were primarily attributable to poor cone handling techniques.

RÉSUMÉ

Des cônes d'*Abies amabilis*, de *Picea glauca*, de *P. sitchensis*, de *P. Engelmannii* et de *Tsuga heterophylla* ont été placés dans des abris ou dans des entrepôts réfrigérés (2°C) pendant diverses périodes allant jusqu'à six mois. Chaque mois, on a retiré des échantillons de cones dont on a fait germer les graines pour en déterminer la viabilité. Après six mois, on n'a trouvé aucune différence de qualité entre les graines entreposées dans les abris ou au froid. Il y a eu quelques cas de viabilité réduite après entreposage, mais ils étaient surtout dus à une manutention imparfaite des cones.

INTRODUCTION

In British Columbia, cones of most species mature in late summer or early fall. Since natural seed fall can occur as soon as one to two weeks after maturity, cones must be picked as soon as they are mature if seeds are to be collected before the cones open. After picking the cones are usually partially dried in the field before they are shipped for extraction. Warm, moist conditions can be deleterious to cone and seed quality (Rediske & Shea, 1965), thus cones must be at least surface dried to reduce deterioration and respiratory overheating during transport.

Upon arrival at the extractory cones are stored in outdoor cone sheds until they can be processed. In heavy crop years cones may be stored for many months before the seeds can be extracted. Because it is not usually possible to process all cones as soon as they are received, different species are roughly ranked according to the relative ability of their seeds to withstand prolonged storage within the cones. For example, at the B.C. Ministry of Forests Duncan Seed Centre cones of ***Tsuga heterophylla*** are extracted first while cones of ***Abies amabilis*** are extracted last. However, the question of how long seeds can be safely stored in cones has not been systematically investigated, so the order in which cones are presently extracted is based primarily upon intuition and experience. Studies of Douglas-fir by Lavender (1958), Rediske (1961), and Rediske & Shea (1965) found that seeds could be safely stored in the cone for up to four months. In some cases seed quality improved after cone storage, probably because cones had been harvested before the seeds were mature (Bloomberg, 1969; Rediske & Nicholson, 1965; ***Picea glauca***, B.S.P. Wang[1] and D.G. Edwards[2], personal communications). Nevertheless, it has not been established whether mature, fully developed seeds of various other conifers can withstand prolonged cone storage without losses in seed viability.

The following study was undertaken to determine how the quality of fully developed seeds was affected by cone storage periods up to six months. Two different storage conditions were compared: (1) operational storage (no environmental control) in covered outdoor sheds, and (2) controlled storage in refrigerated coolers. Five species were chosen for investigation: ***Abies amabilis, Picea engelmannii, P. glauca, P. sitchensis*** and ***Tsuga heterophylla.*** Most of the cones were collected and stored by the British Columbia Ministry of Forests (BCMF), but two commercial cone dealers also cooperated in the study.

MATERIALS AND METHODS

Sampling

Cones were randomly sampled from bulk collections as the lots arrived at the BCMF Duncan Seed Centre. After samples had been withdrawn from several lots, the cones were thoroughly mixed so that the consolidated samples would include representatives from the total geographic and altitudinal range of the original collections. No attempt was made to analyze seed lot variation separately since the intention was to test overall species response, rather than test the response due to local variation.

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[1] Canadian Forestry Service, Petawawa National Forest Institute.

[2] Canadian Forestry Service, Pacific Forest Research Centre.

Storage

Samples were withdrawn from the aggregate sample and divided into separate portions for the two storage treatments. Cone samples to be stored under standard operational conditions were loosely packed in fresh burlap bags and placed on racks in a covered outdoor cone shed. The bags were located away from the outer edges of the shed to avoid exposure to extreme fluctuations in temperature and humidity. The cones for controlled storage were put in cloth mesh bags, placed on top of screen trays to ensure good air circulation, and kept in refrigerated coolers at 2°C. Records of temperature and relative humidity were kept for both storage conditions (Table I).

The two cooperating commercial dealers also stored their cones in burlap bags in covered outdoor sheds. Random samples were sent monthly to the Seed Centre for extraction and testing. Although one storage facility was located on the coast and the other was in the interior of British Columbia, both locations experienced very mild conditions in the winter of 1979. No frosts occurred until November and in only a few instances did temperatures reach as low as -5°C.

Testing

Commencing at the end of October and continuing at monthly intervals until March, cone samples sufficient to yield at least 1000 seeds per species were withdrawn from each storage condition. The cones were loosely packed in mesh bags for kilning, after which they were extracted using a small lot tumbler.

Filled seeds were identified by x-ray, then separated into six 100-seed lots per treatment. For stratification, seeds were soaked for 24 hrs at 20°C, then transferred to capped vials for chilling at 2°C. *Picea* seeds were stratified for 21 days and *Abies* and *Tsuga* seeds for 28 days. Seeds were sown on top of moist blotter paper in covered clear plastic dishes, then germinated under 8 hr light at 30°C and 16 hr dark at 20°C. (*Tsuga* received 20°C during light and dark periods.) Germinants were counted when radicles were 4 times the length of the seed coat (A.O.S.A., 1978; I.S.T.A., 1976). Abnormal germinants were noted separately. Final counts were taken after 21 days for *Picea*, and 28 days for *Abies* and *Tsuga*.

Records

In addition to germination sheets, records were kept of storage treatments and general condition of the cones (Tables I and II). Temperature and relative humidity were continuously monitored for both storage treatments. Condition of the cones was recorded monthly when cones were withdrawn from storage, and factors such as the presence of insects and disease (rust, mold) and degree of cone opening or disintegration were rated. Moisture content samples were withdrawn simultaneously with cone samples for monthly germination tests. Five cones per treatment were taken from various positions throughout the cone sack, and a single moisture content was determined on the composite sample by comparing the total weight of 5 fresh cones with the total weight of cones dried for 24 hrs at 100°C.

RESULTS

Abies amabilis (Amabilis fir)

Germination of *Abies amabilis* seeds was found to fluctuate depending upon whether cones were kept under refrigerated or outdoor storage (Figure 1). The poor condition of the cones when received at the extractory (Table II) probably accounted for most of the variation observed in germination tests. Three of the four lots were moldy when received at the Seed Centre. Heat damage was also suspected in one lot because the cones had overheated due to lack of ventilation. In two of the lots, seeds were found to be poorly developed. The suggestion of immaturity is substantiated by the fact that *Abies* seeds have been shown to benefit from afterripening treatments (Rediske & Nicholson, 1965), and that germination was greater in November than in October. However, after November, deterioration due to the poor initial condition of the cones negated potential benefits from afterripening in storage. It has previously been assumed that cones which have not been carefully handled in the field and during transport will not maintain viability during extended storage. The data in Figure 1 seems to support this assumption.

Controlled storage did not appear to offer any advantage over outdoor storage. Although germination of seeds from controlled storage was slightly higher during the first 3 months, seed quality during the last 3 months of storage was lower than that of seed stored outside. However, reduced germination may have been caused by factors other than storage condition. Even though temperatures fluctuated only slightly during the 6-month period, equipment malfunction caused temperatures to drop on three different occasions between 16-23 January to -9°C, -2°C, and -6°C (Table 1).

Germination results from the commercial dealer were generally high and, with the exception of the second month of storage, appeared to increase with longer storage. As noted in the extractory report (Table II), cones received from

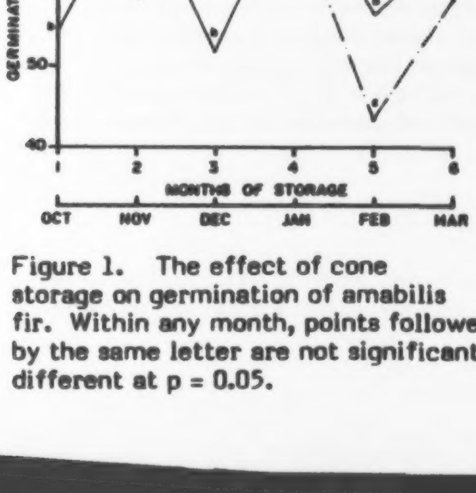

Figure 1. The effect of cone storage on germination of amabilis fir. Within any month, points followed by the same letter are not significantly different at p = 0.05.

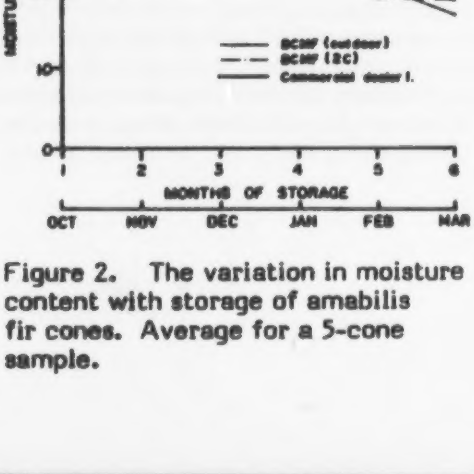

Figure 2. The variation in moisture content with storage of amabilis fir cones. Average for a 5-cone sample.

Table I: **Temperature and Relative Humidity recorded at B.C. Ministry of Forests Seed Centre, Duncan, British Columbia**

A.

OUTDOOR CONE SHED

Period		Temperature (°C)		Relative Humidity (%)	
		Maximum	Minimum	Maximum	Minimum
October	1-15	22	2	96	42
	16-31	17	0	94	52
November	1-15	15	-5	95	52
	16-30	11	-5	95	53
December	1-15	13	-3	100	36
	16-31*	12	-2	97	45
January	1-15	11	0	94	32
	16-31	15	-6	99	17
February	1-15	13	-2	96	34
	16-28	12	-3	96	30
March	1-15	18	2	95	37
	16-31	16	2	97	23

*Flooded 14-22 December

B.

REFRIGERATED STORAGE

Period	Temperature (°C)		Relative Humidity (%)	
	Maximum	Minimum	Maximum	Minimum
October	+1	-1.5	99	78
November	+2	-1.0	100	76
December	+2	0.0	97	74
January*	+2	0.0	92	75
February	+4	0.0	96	80
March	+4	0.0	95	81

*Equipment malfunction, -9°C (16 Jan); -2°C (18 Jan); -6°C (23 Jan)

Table II: Cone Lot Evaluation, Ministry of Forests Seed Centre, Duncan, B.C.

Lot, Spp[1] Identity	General cone Condition	Insects	General seed[2] Condition	Yield (kg seed/ hl cones)
Aa-1	Moldy, damp	Midge, cone moths	Many immature	2.26
Aa-2	Moldy	-	Great variation in maturity; many poorly developed	2.67
Aa-3	Good	-	High volume empty seed; many 'corky'	0.76
Aa-4	Moldy, warm	Heavy activity esp. *Barbara* spp.	Shrunken endosperm, spongy texture	1.03
Commercial Dealer	Good, clean dry	Some present	-	1.37
Sw-1	Heavy mold	Moderate *Laspyresia* activity	-	0.90
Sw-2	Heavy mold, case hardening	-	-	0.47
Sw-3	Sacks damp and overfilled, 30% collection with heavy mold	-	Some endosperms grey and spongy	1.03
Sw-4	Moderate debris, mold, case hardening	Moderate activity and damage	Some immature seed	0.70
Commercial Dealer	Wet, slightly moldy	-	-	1.09
Ss-1	Light mold	-	Some immaturity, 20% of filled seeds have grey and spongy endosperm, variable embryo elongation	0.60
Ss-2	Sacks overfilled	Moderate activity and damage	Some immaturity	0.37
Ss-3	Good	-	-	0.69
Ss-4	Good	-	-	0.54

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[1] Species abbreviations = Aa, *Abies amabilis;* Sw, *Picea glauca;* Ss, *Picea sitchensis*

[2] Based upon cutting tests and x-rays

the commercial dealer were clean, dry, and free from mold. The superior performance of these cones demonstrates how proper handling during early stages of collection and processing can affect subsequent seed quality and performance.

Moisture contents of cones stored outside slowly decreased throughout the 6-months' storage (Figure 2). Moisture contents in both the BCMF and commercial dealer's cones were about 40% in October and dropped to about 20% by March. However, cones in controlled storage remained fairly constant at 20% moisture content during the entire storage period, the only exception being the slightly higher values observed during December.

Picea glauca (White spruce)

For both BCMF cone treatments, storage periods up to 6 months had little adverse effects on germination (Figure 3). Cones stored under controlled conditions appeared to produce better quality seeds overall, whereas seed quality of outdoor-stored cones tended to fluctuate. Cones stored outdoors by the commercial dealer also exhibited considerable variation and generally lower seed quality than the two BCMF treatments. Differences in mold or insect activity did not seem to be the cause of the poor performance (Table II), but since the dealer's cones were generally wetter than the BCMF cones they could have been more susceptible to cold injury (Figure 4). However, the Cone Lot Evaluation (Table II) notes that the dealer's cones were wet and slightly moldy when received at the Seed Centre, which indicates that poor initial handling was also a contributing factor.

Picea sitchensis (Sitka spruce)

Germination in March from cones stored outdoors by BCMF and both commercial dealers was high, demonstrating that prolonged outdoor storage of seeds in cones is not detrimental to final seed quality (Figure 5). In spite of month-to-month fluctuations, tests of samples from the two dealers remained around 70% and exhibited no significant differences (p=0.05) over the 6 months. However,

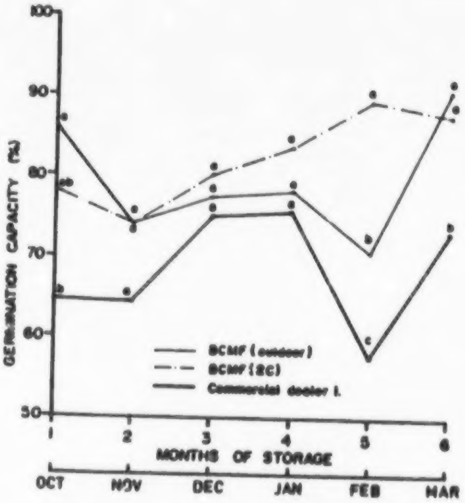

Figure 3. The effect of cone storage on germination of white spruce. Within any month, points followed by the same letter are not significantly different at p = 0.05.

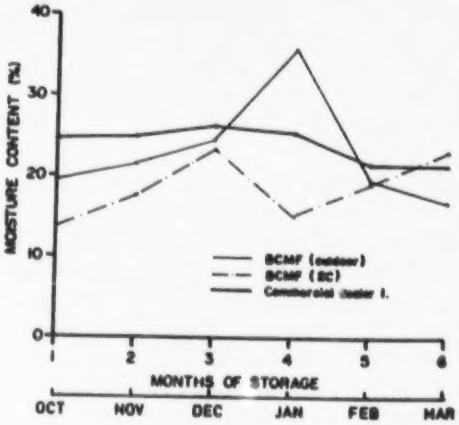

Figure 4. The variation in moisture content with storage of white spruce cones. Average for a 5-cone sample.

BCMF cone collections did not perform as well. Germination tests averaged about 60% and were performed on a mixture of 4 lots, 2 of which were of questionable quality (Table II). Mixing the poor lots with the lots received in good condition apparently resulted in reducing the storability of all cones in the collection. Both poor lots contained immature seed. In one lot sacks were so full that cones had little room to expand, which would have favoured overheating and mold. Since the moldy cones were subsequently mixed with uninfected lots, the contamination probably spread to the remaining cones.

There is some indication that moisture may also influence viability of seeds during storage in the cones. Germination of seeds from cones in controlled storage was around 75% during the first, second, and sixth months of storage, but dropped to 25%, 50% and 55% during the three intervening months. Moisture contents of cones sampled during the second and third months were relatively high (Figure 6) and may have been partially responsible for the low germination observed in refrigerated storage. By comparison, BCMF and dealers' cones kept in outdoor sheds gradually germinated fairly well. Natural ventilation around the cone sacks most likely improved the drying of cones stored outdoors.

***Picea engelmannii* (Engelmann spruce)**

Germination of Engelmann spruce varied from 60% to 75% during storage, but was significantly higher (p=0.05) at the end of storage (Figure 7). Although the data are limited, it appears the Engelmann spruce may benefit from a period of extended storage prior to extraction. Further study of a number of different seed sources would be required to determine if improved seed quality from prolonged storage is a generalized response.

Cone moisture content did not change appreciably from October to March, staying relatively low around 15%.

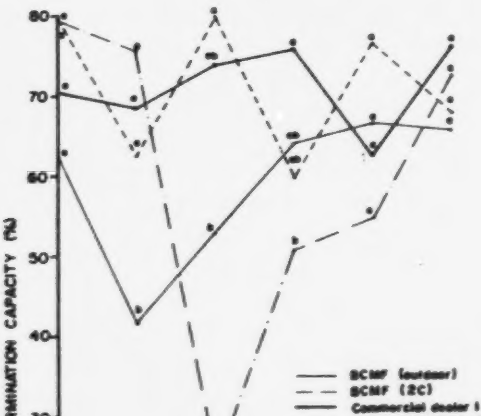

Figure 5. The effect of cone storage on germination of sitka spruce. Within any month, points followed by the same letter are not significantly different at p = 0.05.

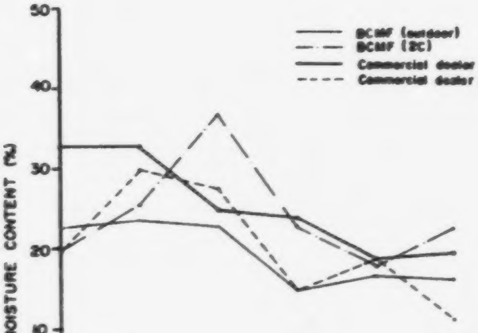

Figure 6. The variation in moisture content with storage of sitka spruce cones. Average for a 5-cone sample.

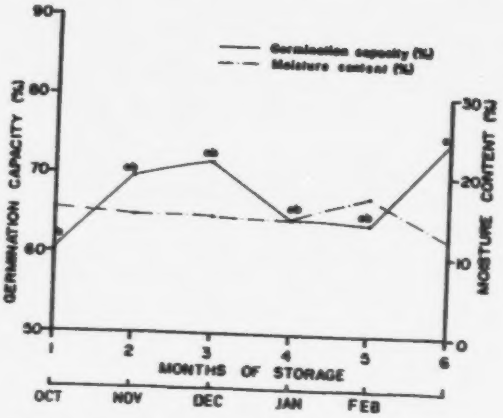

Figure 7. The effect of cone storage on germination and moisture content of Engelmann spruce. Germination data points followed by the same letter are not significantly different at p = 0.05. Moisture contents represent an average for a 5-cone sample.

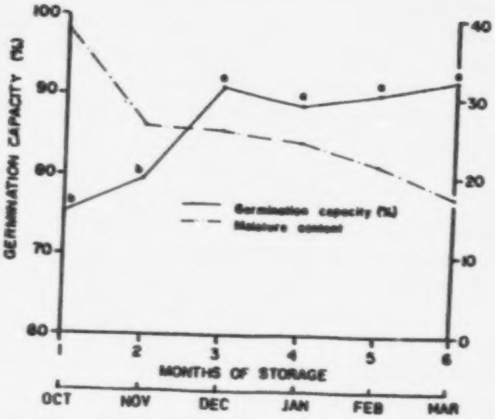

Figure 8. The effect of cone storage on germination and moisture content of western hemlock. Germination data points followed by the same letter are not significantly different at p= 0.05. Moisture contents represent an average for a 5-cone sample.

Tsuga heterophylla **(Western Hemlock)**

Germination of western hemlock also increased significantly (p=0.05) from 75% in October to 91% by December, and remained at high levels until completion of storage in March (Figure 8).

Initial moisture levels for western hemlock were high, reaching almost 40%, but dropped sharply after October and declined steadily until approaching a level of 17% in March.

DISCUSSION

Germination data were sometimes difficult to interpret because some of the variation appeared to be due to differences in the handling and quality of the cones rather than differences due to storage. The methodology for this study was to combine all the cones for each species into a single composite lot, from which monthly samples would be withdrawn for germination tests. But storage effects would have been easier to distinguish from handling and source effects if the cones and data for each lot had been kept separate during the experiment, then combined later for purposes of analysis. With this revision, species response to storage could have been studied apart from other factors which were more specific to individual lots.

However, the significant variables affecting storability of cones can still be identified. The important factors appear to be seed maturity at the time of collection and the manner in which cones are initially handled, especially during interim field storage and transport to the extractory. Based on comments from the Seed Centre Report (Table II) and the germination results (Figures 1,3,5,7,8), cones which were poorly developed or improperly handled did not store well regardless of subsequent storage conditions. But if cones were handled in accordance with good practice, germination capacity of ***Abies amabilis, Picea sitchensis, P. engelmannii,*** and ***Tsuga heterophylla*** was maintained or increased with longer storage.

There were only two instances in which prolonged storage adversely affected seed quality. In refrigerated storage both ***Abies amabilis*** and ***Picea sitchensis*** had lower germination in March than in October (Figures 1,5). Generally ***Abies*** seed benefits from storage prior to extraction. Even after seeds are completely developed ***Abies*** seem to require additional time to complete after-ripening and ensure good germination (Ching, 1960; Rediske & Nicholson, 1965). Rediske & Nicholson speculated that the additional storage was necessary for transfer of materials from the cone to the seed, and advised that ***Abies*** seed be stored at least 4 weeks prior to extraction. Germination results from the commercial cone samples were in agreement with the studies cited above, thus it can be assumed that the lower and variable performance of the two BCMF treatments resulted from other factors, most likely the poor initial condition of the cones (Table II). The fact that the cones submitted by the commercial dealer were dry, clean, and free from mold lends support to the conclusion that properly handled cones will store well.

There was no apparent reason for the deterioration of ***Picea sitchensis*** cones in controlled storage, but it may have been due to immaturity in some of the lots (Table II). There is also evidence that some ***Picea sitchensis*** seed sources are susceptible to fungal attack in cold storage (Sutherland & Woods, 1978) and this may have accounted for the poor performance.

In general, temperature-controlled storage of cones did not appear to offer any advantage over outdoor storage. Germination of ***Abies amabilis, Picea glauca*** and ***P. sitchensis*** was about the same for both storage conditions when averaged over the six-month period (Figures 1,4,6). Outdoor storage may even be superior since natural ventilation tends to promote evaporation and gradual drying, whereas indoor air is not as effective in drawing moist air away from the cones. On the other hand, cones stored outside are subject to low temperature injury, thus local environmental conditions have to be considered if outdoor storage is to be used.

It may be concluded from this study that if stored under proper conditions, seeds may remain in the cones up to 6 months without detrimental effects upon seed quality. However, it should be emphasized that initial handling is extremely important. Cone collectors should make special efforts to provide temporary storage facilities close to collection sites so that cones are dried in the field and are not shipped in a moist condition. At the extractory cones must also be kept dry and well ventilated. An open shed with a roof and well-spaced racks should be suitable for maintaining seed quality during most prolonged storage.

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EFFECT OF FREEZING TEMPERATURES ON NOBLE FIR SEED DURING CONE STORAGE

Yasuomi Tanaka
Weyerhaeuser Western Forestry Research Center
Centralia, Washington 98531
U.S.A.

ABSTRACT

Noble fir (*Abies procera* Rehd.) seed in intact cones prior to processing experienced below-normal freezing temperatures as low as -20°C in a cone shed located near Olympia, Washington, USA, during December 1978 and January 1979. A laboratory seed test following the freeze indicated that viability had been adversely affected by the cold weather. Subsequently, laboratory freezing tests were conducted to determine cold tolerance using processed noble fir seed. Results showed that the 50 percent kill temperatures (LT_{50}) were -32°C and -12°C for seed with 20 percent and 35 percent seed moisture contents, respectively. Results of laboratory freezing tests with intact noble fir cones were generally in agreement with those with processed seed. The 10-percent kill temperatures (LT_{10}) estimated from the above data for seed in intact cones suggest that frost protection measures are necessary to avoid embryo damage during relatively mild frosts.

RÉSUMÉ

En décembre 1978 et en janvier 1979, des graines de sapin noble d'Amérique (*Abies procera* Rehd.) contenues dans des cônes intacts et non traités ont subi des températures sous la normale (-20°C) alors qu'elles étaient entreposées dans un abri pour cône situé près d'Olympia, (Washington) aux Etats-Unis. D'après un test subséquent en laboratoire, le temps froid a eu un effet néfaste sur la viabilité des graines. Par la suite, on a effectué des tests en laboratoire en vue de déterminer la tolérance au froid des graines traitées. Les résultats ont montré que les températures qui ont provoqué 50% de mortalité (LT_{50}) ont été respectivement de -32 et de -12°C pour les graines ayant 20% et 35% d'humidité. Dans l'ensemble, les résultats d'autres tests de congélation effectués sur des cônes intacts correspondaient à ceux qui ont été obtenus avec les graines traitées. A partir des données précédentes, les temperatures qui provoqueraient 10% de mortalité (LT_{10}), ont été estimées pour les graines contenues dans des cônes intacts, et il en ressort que des mesures de protection contre le gel seraient nécessaires pour éviter les dommages causés à l'embryon au cours de gels relativement légers.

INTRODUCTION

In large-scale collections, harvested cones are often stored for a prolonged period under outdoor conditions. These cones are subject to freezing temperatures in late fall and early winter. This is particularly true with noble fir (*Abies procera* Rehd.) because cones are harvested at high elevations relatively late in the season and seed requires a 1-2 month after-ripening storage period before processing to attain maximum germination potential (Rediske and Nicholson 1965).

In 1978 there was a good crop of noble fir cones in the western Cascade Mountains in the state of Washington U.S.A. A large number of cones were harvested and stored through the winter. Some unprocessed cones of noble fir stored in an outdoor covered shed experienced unusually cold weather at a seed plant in Rochester, Washington, near Olympia. The daily minimum temperatures during this period were between -5°C and -20°C (Figure 1), and most cones remained frozen until late January 1979 under the outdoor shed. Germination tests of seed extracted prior to and after the freeze suggested that seed had sustained freezing injury resulting in as much as 60-percent reduction in germination (Figure 2).

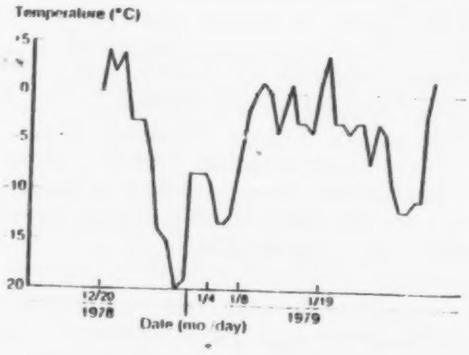

Figure 1. Daily minimum temperatures recorded at the Rochester seed plant near Olympia, Washington, USA.

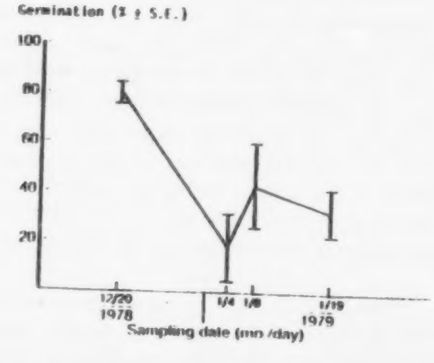

Figure 2. Germination potential of two to eight lots of noble fir seed sampled on four different dates.

While some work has been done on the effect of cold temperatures on flower crops or conelets in conifers (Barras and Norris 1964, Krugman 1966, Simak 1972, Sorensen and Miles 1974, Timmis 1977; Timmis and Dorn, unpubl. data[1]) little is known about the impact of freeze damage on mature seed in harvested cones prior to seed extraction. The present study was therefore conducted to measure the cold hardiness of harvested noble fir seed and determine the level of protection required during cone storage. Special consideration was given to seed moisture content because observations during the 1978-79 freeze suggested that freezing injury was strongly dependent on this factor.

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[1] Weyerhaeuser Western Forestry Research Center. Centralia, Washington, USA

MATERIALS AND METHODS

The study was conducted in two phases. The first phase involved the freezing of seed that had been collected, extracted and stored since 1974. The purpose of the seed-freezing test was to estimate, on a preliminary basis, the impact of seed moisture content on cold hardiness of noble fir seed. This phase of the study provided supporting data for the second phase, which involved the freezing of seed in intact cones. Seed moisture content was controlled in the seed freezing test, but this was not possible in the cone freezing tests. Therefore, seed moisture content was measured following these latter tests and regression analysis used to determine moisture's effect on freeze damage.

Seed Freezing Tests

Seed used in these tests had been collected at 1000 m elevation in the Snoqualmie area (latitude 47°30' and longitude 121°50') near Seattle, Washington in 1974, dried to 9-percent moisture content (fresh weight basis) and stored at -18°C. It was then soaked in water for 24 h and air-dried, to 20-percent and 35-percent seed moisture contents (fresh weight basis) at room temperature (~27°C, 30-40 percent relative humidity). This moisture adjustment was done on the basis of the precalculated moisture content/seed weight relationship obtained using different sub-samples of the same seedlot. The moisture levels to which seed was dried (20% and 35%) represented the ranges of seed moisture content encountered in different parts of gunny sacks sampled in January 1979. Seed with the two different moisture levels were then subjected to freezing tests at -5°C, -20°C and -35°C. Seed was placed in plastic bags and cooled conductively at the rate of 5°C/h until a desired temperature was reached, left at that temperature for 2 h, and then warmed at 20°C/h. The controls for both seed moisture contents remained unfrozen at 4°C until the freezing tests. There were three replications of 50 seeds in each moisture/temperature combination. Following the freezing tests seed viability was tested using the modified Association of Official Seed Analysts testing rules (1978). Seed was stratified for 14 days, placed on vermiculite (substrata) moistened to 80 percent of its water holding capacity, and germinated in an alternating environment of 20°C for 16 h in the dark and then 30°C for 8 h in the light. Seed was considered germinated when a radicle grew to the same length as the seed coat.

The percentage of seed germination in each replication was calculated from the predetermined number of filled seed. The percentage germination at each temperature was divided by that of control seed to estimate the percentage live seed. The data were analyzed by analysis of variance after arcsin transformations. The differences among means were tested by Duncan's multiple range test. To estimate the 50 percent kill temperatures at each seed moisture level, the live seed ratios were transformed into probits (Finney 1971) and the linear regression equation describing the relationship between the probits of live seed (Y) and temperature (X) was computed.

Cone Freezing Tests

The noble fir cone crop was light in 1979 and it was not possible to obtain experimental materials from various geographical sources. Only limited numbers of cones were available near Snoqualmie, Washington, in early September. They were stored in gunny sacks under a covered shed at the Rochester seed plant for 2 months before the tests. Sample cones were drawn from the sacks in late November, transferred into smaller plastic mesh bags, and frozen (subjected to the freezing tests) at -5°C, -20°C and -35°C, and tested using the same procedure as for the seed freezing tests. Unfrozen cones served as controls. Eight cones were tested at each freezing temperature.

Immediately after the freezing tests 10 seeds were extracted manually from each cone and their moisture contents determined after drying at 105°C for 24 h. The remaining seeds were cleaned and tested for germination using the same procedure described above. A moisture determination conducted with several cones indicated that there was no change in seed moisture content during the freezing tests. The live seed ratios, calculated as in the seed freezing tests, were transformed into probits and the regression equation relating probits of live seed (Y) to temperature (X_1) and seed moisture content (X_2) was used to estimate the 50 percent kill temperatures at different seed moisture levels.

RESULTS

Seed Freezing Test

The effect of freezing was highly dependent upon seed moisture content. Germination of seed with 35-percent moisture content was reduced by 62 percent (significant at $\underline{P} < 0.05$) at -20°C, while it was reduced by only 11 percent (not significant $\underline{P} < 0.05$) when seed moisture content was 20 percent (Table 1). Freezing at -35°C severely reduced germination of seed (by > 50 percent) at both moisture contents. Even at -5°C, germination was reduced by 7 percent and 10 percent (for 20 percent and 35 percent moisture content, respectively) compared with the unfrozen controls. These latter reductions, were not statistically significant ($P < 0.05$), however.

Table 1. Effect of freezing temperature on germination percentage of noble fir seed at two seed moisture levels.

Freezing Temperature (°C)	Seed Moisture Content[1] 20% (% SE)	35% (% SE)
-5	69 ± 5 de[2]	59 ± 5 d
-20	65 ± 8 de	7 ± 2 b
-35	25 ± 11 c	0 a
control	76 ± 4 e	69 ± 10 de

[1] On a fresh weight basis.

[2] Values followed by the same letters are not significantly ($\underline{P} < 0.05$) different as determined by the Duncan's multiple range test.

After probit transformation of live seed ratios for all three temperatures studied, the linear regression equations relating the probits (Y) and temperature (X) were $Y = 7.01 + 0.064\,X$ ($r^2 = 0.69$) for 20-percent seed moisture content and $Y = 6.73 + 0.14\,X$ ($r^2 = 0.98$) for 35 percent seed moisture content. The 50-percent kill temperatures (LT_{50}) estimated from these equations were -31.6°C and -12.4°C for 20 percent and 35-percent seed moisture contents, respectively.

Cone Freezing Tests

The regression equation, after probit transformation of the live seed ratios, was $Y = 8.55 - 0.0087X_1 - 0.0324X_2 + 0.0047 X_1 X_2$ ($r^2 = 0.53$). The 50 percent kill temperatures estimated from this equation were -34.1°C and -14.7°C for 20 percent and 35 percent moisture content, respectively.

DISCUSSION

The results of the cone freezing tests were in agreement with those of the seed freezing tests in that susceptibility to a given freezing temperature increased as seed moisture contents increased. The probit regression equations established by the two freezing methods were similar at both 20-percent and 35-percent seed moisture contents (Figure 3). The slopes and intercepts of the equations were not significantly ($P < 0.05$) different, but slopes were slightly greater in the cone-freezing tests than in the seed-freezing tests. This is probably due to the insulating effect of cone scales. As a result of this effect LT_{50} temperatures were slightly lower in the cone freezing tests than in the seed-freezing tests at both 20-percent (-34.1°C vs -31.6°C) and 35 percent (-14.7°C vs -12.4°C) seed moisture content.

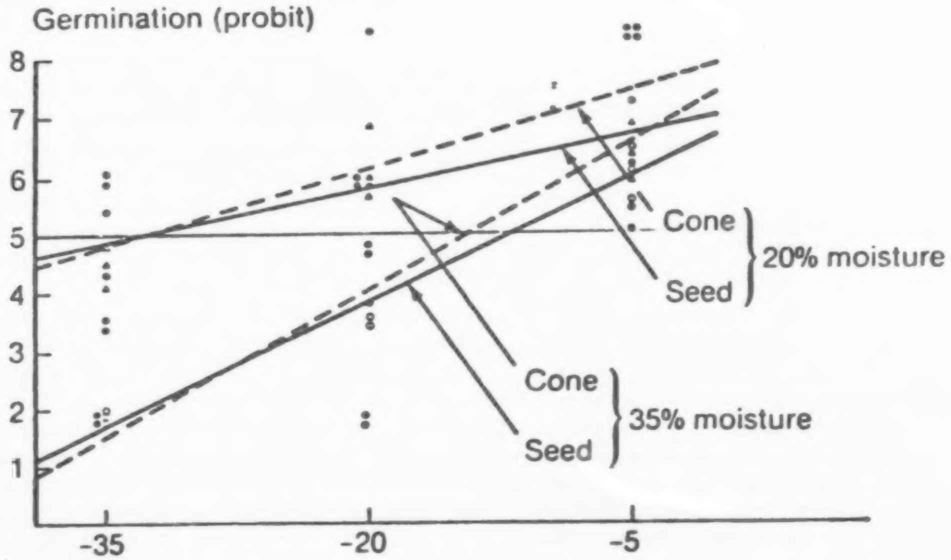

Figure 3. Regression lines showing the relationship between live seed (probit) and freezing temperatures in cone and seed freezing tests at two seed moisture levels: ● denotes data points for cone freezing; △ and ○ denote data points for seed freezing at 20% and 30% seed moisture contents, respectively. The 50% kill temperatures (LT_{50}) are the intercepting points on the regression lines by probit value 5.

The cone freezing study showed that, at 35-percent seed moisture content, more than 50 percent of seed can be damaged at only -14.7°C, a temperature milder than the minimum (-20°C) observed during the 1978-79 winter. On the other hand, a considerably more severe frost (-34.1°C) was required to kill 50 percent of seed at 20-percent seed moisture content. These results further emphasize the importance of cone drying during storage. In addition to its previously known benefits -- i.e., effective after-ripening and efficient seed extraction -- cone drying reduces frost damage.

For cone protection during the post-harvest storage period, it would be more important that a lower kill temperature, such at LT_{10} (10 percent kill temperature), be determined for practical purposes. The LT_{10} values estimated from the probit equations were -19.2°C and -6.9°C at 20-percent and 35-percent seed moisture content, respectively, in the cone-freezing test. The observations during the study showed that seed in the centers of gunny sacks usually had a moisture content around 35 percent. Therefore, given the above LT_{10} (-6.9°C) at this moisture level, it would be necessary that adequate frost protection measures be provided for storage of noble fir cones during frost seasons because of the following four considerations: (1) the freezing temperatures that would kill 10 percent and 50 percent of seed with 35-percent moisture content occur with relatively short return periods of less than 5 and 10 years during the winter, respectively, in Olympia, near the seed plant where the study was conducted (Dunham, unpubl. data [2]); (2) in contract to seedlings older than 6 weeks, embryos of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) are not capable of developing freezing tolerance prior to germination even after being exposed to cold temperatures (Timmis 1975), and this is probably true with conifers of other genera as well; (3) seed is often exposed to freezing temperatures more than once during the storage period and repeated freezings can cause greater embryo damage than a single frost (Tanaka, unpubl. [3]); and (4) the duration of the freeze period is often longer than the 2 h used in the present study, and such prolongation during natural frosts could result in greater damage than that observed.

Although limited number of samples were available in the cone freezing tests in this study, the results do suggest that 10-percent seed damage can be brought about by a relatively mild frost (-6.9°C) when seeds have a high moisture content. Additional tests are necessary to determine variations in cold tolerance of noble fir seed in intact cones within and among different geographic sources.

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[2] Information derived from climatological data 1948-1974, summarized by National Oceanic and Atmospheric Administration, U.S. Dept. Commerce, Washington, DC, USA.

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GERMINATION AFTER TWO YEARS STORAGE OF ARTIFICIALLY RIPENED WHITE SPRUCE SEED

by B.D. Haddon and D.A. Winston
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, K0J 1J0

ABSTRACT

Twenty white spruce seedlots that had been artificially ripened in cones at 5°C, were re-examined after 2 years of seed storage. Germination tests indicate that mature, artificially ripened seeds, or seeds from cones stored at 5°C, can be stored at least 2 years and still retain viability. In previous findings, cone storage at 5°C eliminated dormancy in white spruce seeds. This non-dormant condition was also found to be retained in the seeds after 2 years of storage. Of the seedlots tested, 80% had germination of non-stratified seeds virtually equal to or in excess of fresh seeds. Ten per cent of the seedlots were immature at time of extraction and dropped in non-stratified germination. Only 10% of the seedlots showed a reduction in non-stratified germination that may be attributable to sampling bias or poor seed storage. Stratification of the stored seeds reduced germination, possibly indicating that the seed vigor decreased as a result of poor seed storage.

RÉSUMÉ

Vingt-neuf lots de graines d'épinette blanche, qui avaient été artificiellement mûries à 5°C dans leurs cônes, ont été réexaminées après un stockage de 2 ans. Les tests de germination indiquent que des graines matures artificiellement mûries ou provenant de cônes stockés à 5°C peuvent être conservées pendant au moins 2 ans sans perdre de leur viabilité. Auparavant, on avait découvert que le stockage des cônes à 5°C éliminait la dormance chez les graines d'épinette blanche. On a remarqué que cette condition de non-dormance se maintient dans les graines après un stockage de 2 ans. Quatre-vingts pour cent des lots de graines non stratifiées possédaient un taux de germination virtuellement égal, sinon supérieur à celui des graines fraîches. Au moment de leur extraction, 10% des lots de graines n'étaient pas à maturité et se sont affaissées pendant la germination non stratifiée. Seulement 10% des lots ont présenté une réduction de germination non stratifiée que l'on pourrait attribuer à un échantillonnage biaisé ou à un mauvais stockage. La stratification des graines stockées a réduit la germination, ce qui pourrait indiquer que la vigueur des graines s'est affaiblie par suite d'un mauvais stockage.

INTRODUCTION

The extension of the cone collection season using artificial ripening techniques has been examined for several species (Silen 1958; Rediske 1961; Waldrip 1970; Zasada 1973; Oliver 1974; McLemore 1975; Barnett 1976; Edwards 1977, 1978). In 1978, cones of six white spruce (*Picea glauca* (Moench) Voss) trees were collected at intervals through the summer, commencing in mid-July, and subjected to a variety of storage conditions and periods to determine whether the seeds could be artificially ripened.

Results indicated that eastern Canadian white spruce cones could be collected as early as August 1 and stored to produce viable seeds (Winston and Haddon 1981). The optimum treatment for that date was storage on screened trays at 5°C with high relative humidity for a 12 week period. Cold storage of cones, regardless of collection date, eliminated the need for stratification of seeds which was otherwise required to obtain high germination. Such results have important ramifications in the understanding of stratification dormancy relationships and have future potential in forestry operations. However, it was uncertain whether seeds obtained from cones subjected to cold storage retained their viability and non-dormant condition over a period of seed storage. A study was initiated to test the germination of some artificially ripened seeds after two years of seed storage.

METHODS

In 1978, cones from six white spruce trees at the Petawawa National Forestry Institute (45°58'N; 77°25'W) were collected on July 11, July 18, August 1, August 15 and in late August, immediately before seed dispersal from each tree. The final collection was actually made from August 23 to August 31, but has been designated as August 29 for convenience. Some cones from each collection were subjected to immediate extraction and the remainder were stored under various conditions for 4, 8 or 12 weeks. Two storage temperatures, viz., 5°C in a controlled temperature chamber (relative humidity 75-95%) and ambient air temperatures in a cone storage building were used with the cones exposed to the air on screen-bottomed trays or in plastic bags with moist peat moss.

For seed extraction, the cones were placed in a cross-circulation kiln for 16 hours at 49°C and 45% relative humidity. Some cones, particularly from early collections, failed to open fully and seeds had to be extracted by hand. The seeds were dry-dewinged by rubbing in a cotton bag and cleaned in an air separator.

Germination was tested at 30°C for 8 hours with light and 20°C without light for 16 hours using moist cellulose wadding as the germination medium. As far as possible, four replications of 100 seeds each were used in each test, but where there were insufficient seeds replications were reduced to 25, 50 or 75 seeds. Tests were conducted on two samples from each seedlot, one with and one without a 21-day period of moist prechilling (stratification) at 5°C. Seedlings were recorded as germinants if, after 21 days germination, the primary root and hypocotyl together exceeded four times the length of the seed, provided all structures which had developed appeared normal. Germination percentages were based on filled seeds.

Seeds not used in the initial germination tests were placed in paper envelopes which were wrapped in plastic and stored at 1°C. Storage conditions were less than optimal as the seedlots were retrieved from cold storage on a number of occasions and exposed to warm temperature and high humidity.

In August 1980, approximately 24 months after collection, germination of 20 seedlots from the cold-air cone storage treatment was re-tested. The cold-air cone storage treatment was chosen since it was the treatment that provided both excellent seed germination of early cone collections and eliminated seed dormancy and need for stratification. Not all seedlots were represented due to insufficient seed quantities, and even for those tested the quantities of seed available did not permit fully replicated tests, some tests consisting of only one replication of 25 seeds. Otherwise, the germination tests were conducted as described above.

Germination results for each seedlot from both the 1978 test of fresh seeds and the 1980 re-test of stored seeds (both non-stratified and stratified) were adjusted using the standard arcsin transformation. The differences between stored (stratified) vs. fresh (stratified) and between stored (stratified) vs. stored (non-stratified) were then tested using t-tests (Table 1).

RESULTS AND DISCUSSION

"Non-Stratified" Seed Germination

After storage for 2 years, 80% of the 20 seedlots were able to germinate immediately, without stratification, virtually as well as or better than when fresh (1978 test) (Table 1). Those seedlots which increased germination in the 1980 test may have undergone a type of after-ripening. Of the remaining four seedlots that exhibited a reduced level of germination compared to fresh, two (August 1, trees 3 and 4, 4 weeks of cone storage) were of very poor initial (fresh) germination, suggesting that the seeds were immature at the time of extraction. For example, seeds from cones of tree 3 collected on August 1 and stored in the cones for 4 weeks germinated poorly in 1978. After 2 years of seed storage the germination of this seedlot dropped indicating that ripening in the cones had not been completed. Conversely seeds from cones from tree 3 collected on August 1 and subjected to 12 weeks of cone storage had higher germination than those subjected to 4 weeks of cone storage in 1978 and even increased considerably after 2 years of seed storage. Seeds on other trees, e.g. 2 and 6, had evidently attained a degree of development by August 1 which allowed maturation in 4 weeks of cone storage.

The cause for reduction in germination of the other two seedlots (August 29, trees 2 and 6, 12 and 8 weeks of cone storage respectively) is unknown but may be related to sampling bias and poor seed storage conditions (e.g., excessive seed moisture content) and subsequent handling.

Stratified Seed Germination

For most seedlots tested after 2 years seed storage, germination of stratified samples was considerably lower than that of non-stratified samples. This reduction varied a great deal from one seedlot to another, with several seedlots showing large reductions and others showing little or no reduction, and could not be explained from differences among trees, collection dates or storage periods.

Table 1. **Effect of two-years of seed storage (1°C) on germinability and dormancy of white spruce seed obtained from cones ripened artificially at 5°C for various storage periods.**

Cone Collection Date	Tree No.	Cone Storage Period (weeks)	Germination (%)				t-test[1/]	
			Fresh Seed (1978)		Stored Seed (1980)		Non-Strat. 1980 vs Strat. 1980	Strat. 1978 vs Strat. 1980
			Non. Strat.	Strat.	Non. Strat.	Strat.		
July 18	6	0	0	0				
		12	77	76	100	44	**	**
August 1	2	0	0	0				
		4	79	76	90	38	**	**
	3	0	1	2				
		4	54	48	20	17		
		12	69	76	85	68		
	4	0	1	3				
		4	37	33	17	22		
	6	0	0	0				
		4	92	90	96	77	*	
		8	95	96	92	84		
	8	0	0	0				
		4	72	66	68	55	*	*
August 15	2	0	1	21				
		8	92	92	92	72		*
		12	88	85	94	87		
	3	0	0	4				
		12	79	81	87	88		
	6	0	2	18				
		12	91	93	96	76	*	*
	8	0	5	7				
		12	88	89	85	22	**	**
	9	0	2	41				
		4	79	61	75	80		
August 29	2	0	38	74				
		4	97	94	95	88	*	*
		12	91	87	62	24	*	**
	6	0	15	94				
		8	93	95	80	43		**
	8	0	18	89				
		4	94	93	92	80		
		8	98	98	95	72	*	**
		12	89	87	100	84	**	

[1/] ** = significant at P = 0.01, * = significant at P = 0.05

N.B. Each combination of cone collection date, Tree no. and cone storage period is considered to be a Seedlot.

General Discussion

Based on the results and comments reported above, it is apparent that the viability and elimination of dormancy in the seeds as a result of cold-air storage and ripening of cones is retained even after 2 years of seed storage.

Seed dormancy is a very complex phenomenon and has received much attention in forest research. Dormancy within a species does not always appear to be consistent. Wang (1974) and Zasada *et al.* (1978) have stated that white spruce seed requires stratification whereas Heit (1961) and Hellum (1968) did not feel that it is necessary.

The results reported above indicate that dormancy is affected by cone handling and storage since cold, humid, storage (5°C, 75%-95% R.H.) of the cones prior to seed extraction seems to have eliminated dormancy by satisfying the stratification requirement. It is possible that inconsistencies in work elsewhere may be partially due to cone storage conditions. For instance, a period of cold damp weather during cone storage may provide a natural cold (stratification) treatment. The present results also indicate that once the dormancy has been removed in cone storage, subsequent kiln-drying and seed storage does not initiate a relapse into dormancy.

The reduction in viability of stratified seeds after 2 years of storage may result from unfavourable stress conditions placed on the seeds by stratification, which may cause changes in seed biochemistry, reduce embryo vigor, cause seed aging or actually damage the embryo physically thereby reducing embryo vigor (B.S.P. Wang, personal communication, 1980) If this is true, then, even though high germination occurred in the non-stratified samples, seed quality is suspect and may reflect the poor storage and handling conditions after extraction.

CONCLUSIONS

To the best of the authors' knowledge, no other studies have addressed the question of long-term seed storage effects on germination of artificially ripened seeds. This study strongly suggests that seed viability is retained provided seeds have obtained a critical level of development before extraction. Furthermore, the elimination of dormancy in white spruce seed through the use of cold humid cone storage is also retained during seed storage.

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NATURAL SEED STORAGE IN SEROTINOUS CONES OF *PINUS BANKSIANA*

W.D. Baker

Ontario Ministry of Natural Resources
Ontario Forest Research Centre
Maple, Ontario L0J 1E0 Canada

ABSTRACT

Jack pine (*Pinus banksiana* Lamb.) cones were collected by six cone age classes (1, 2, 3-4, 5-6, 7-9, 10+ years from maturity) to determine the effect of natural seed storage on seed yield and viability. The results indicate that both seed yield and germination decreased with increased age of cone. Abnormal germinants increased with cone age. Seeds from one year old serotinous cones averaged 18.1 filled seeds per cone and 88% germinative capacity and this decreased to 11.8 and 55% respectively for seeds in cones 10 years and older. It is recommended that only cones up to six years of age be collected when prompt, uniform germination is required such as in nursery and container stock production.

RÉSUMÉ

On a recueilli des cônes de pin gris (*Pinus banksiana* Lamb.) pour six classes d'âge (1 an, 2 ans, 3 à 4 ans, 5 à 6 ans, 7 à 9 ans, et 10 ans et plus depuis leur maturation) afin de déterminer l'effet du stockage naturel des graines sur leur comportement et leur viabilité. Les résultats indiquent que la quantité et la faculté germinative des graines diminuaient avec l'âge du cône, tandis qu'augmentait le nombre de plantules anormales. Les cônes sérotinaux d'un an ont donné en moyenne 18.1 graines viables et un taux de germination de 88%, alors que les cônes âgés de 10 ans et plus ont donné respectivement 11.8 et 55%. Il est recommandé de ne cueillir que des cônes de moins de 6 ans si l'on désire une germination prompte et uniforme, comme dans le cas de la production de plants en pépinière ou en contenants.

INTRODUCTION

Jack pine (*Pinus banksiana* Lamb.) is one of Ontario's most important commercial species. Unfortunately after cutting, many jack pine areas have failed to regenerate satisfactorily under natural regeneration systems within a reasonable length of time. Thus there is a need for a massive artificial regeneration program which involves both planting and seeding of jack pine. Since jack pine cones are serotinous (persistent, closed), it is possible to collect cones of all ages throughout the year. However, the yield and viability of the seed in the older cones is questionable. Some researchers have found the yield and viability to decrease with increasing age of cone (Schantz-Hansen 1941; Eyre and LeBarron 1944; Rudolf and Ralston 1953; Beaufait 1959; Roe 1963; Ahlgren 1970), while other investigators disagree (Noakes 1946; Popovich *et al.* 1970).

To clarify this aspect of natural seed storage, a study was undertaken to determine which cone ages should be collected. The results of this study are presented in this paper.

MATERIALS AND METHODS

Site class 1 (Plonski 1974) unmanaged jack pine stands, ranging in age from 28 to 80 years, were located during the summer of 1978 in the Gogama District of northeastern Ontario (47° 50'N, 81° 50'W). Two hundred randomly selected dominant or co-dominant trees were felled from these stands. All mature cones were picked, aged, and assigned to one of the following cone age classes:

Class I - 1 year old cones which matured in the fall of 1977;
Class II - 2 year old cones which matured in the fall of 1976;
Class III - 3 to 4 year old cones which matured in the fall of 1975 and 1974;
Class IV - 5 to 6 year old cones which matured in the fall of 1973 and 1972;
Class V - 7 to 9 year old cones which matured in the fall of 1971 to 1969;
Class VI - cones 10 years of age and older which matured in the fall of 1968 or earlier.

Cone age was determined by counting the nodes or internodes along a branch, as well as counting the annual rings on a cross section just below the cone. For each cone age class from each tree, the cones were counted and the volume determined by water displacement.

Seed Processing

Seed extraction and cleaning was conducted at the seed plant of the National Tree Seed Centre, Petawawa National Forestry Institute, Chalk River, Ontario. Extraction was based upon cone age class per tree, and not by individual cones. The cones were soaked in warm water (27°C) for two hours and then kiln dried at 60°C for 16 hours. All seeds were moist dewinged, cleaned by an aspirator, and conditioned in an air oven for 16 hours at 27°C. Any foreign matter not removed by the aspirator was eliminated by hand cleaning. Seed weight was determined for each cone age class of every tree using 4 samples of 50 seeds each.

Germination Tests

Two replicates of 50 seeds were taken from each of the 6 cone-age classes of 40 trees for seed quality testing. Each tree was randomly assigned to a plastic 33 x 18 x 5 cm germination tray. Within each tray a 50 seed replicate for each of the six cone age classes was placed separately on moist Kimpak germination medium. The seeds were not stratified before testing. The germination trays were covered with saran wrap and placed randomly in a germinator.

Germination conditions were standard ISTA criteria for jack pine (Anonymous 1976): 30°C for 8 hours with light and 20°C for 16 hours in darkness under high relative humidity (90%+). The illuminance was approximately 1,000 lux of fluorescent light. Seeds were considered germinated once the seed coats were raised off the germination medium. Germination counts were recorded every other day for 18 days and germinants removed just prior to shedding their seed coats (day 6-8). Abnormal germinants were evaluated by the criteria established by ISTA (Anonymous 1977). Seeds that failed to germinate at the end of the 18 day test period were cut and examined to determine whether they were empty, dead or fresh ungerminated.

RESULTS

Cone and Seed Characteristics

Jack pine cone colour varied with cone age due to weathering. Immature, ripening cones were green and changed to an olive brown or lustrous tawny yellow colour upon ripening. As cone age increased, a grey tone became increasingly more apparent. Two year old cones exhibited this greyish tinge on the weathered or exposed side. By age seven the cones were an ash or light grey colour on all sides. After ten years, they were a dark grey to black colour with many cones covered with lichens.

Both cone size and seed weight varied with cone age (Table 1) which undoubtedly reflects environmental conditions during cone and seed ontogeny. However, this variation did not occur simultaneously; cone age classes with large cones (III and VI) did not necessarily produce the heaviest seed (II).

The number of filled seeds per cone decreased as the cones became progressively older (Table 1). Filled seeds per cone decreased from a maximum of 18.1 in cone age class I, to a minimum of 11.8 filled seeds per cone in cone age class VI. Initially it was thought that this reduction was a consequence of poor cone opening and subsequent seed release in the older cone age classes during seed processing. Consequently, the six cone age classes for a number of trees were extracted a second time to determine if all filled seeds were recovered from the older cone age classes. The results of this test indicated no statistical differences in the percentage of seed recovered from a second kiln extraction of older jack pine cones. Approximately 95% of all filled seeds were recovered during the first extraction. Some older cones were, in fact, more efficient in releasing seed than young cones, as the dry cone scales broke apart from the cone axis during extraction, thereby releasing nearly all seeds. However, a consequence of this was that more time was consumed cleaning the seed e.g., separating cone scales from seed by screening.

Table 1. **Effect of cone age on jack pine cone and seed characteristics.**

		Cone characteristics			Seed characteristics			
Cone age class	No. of seedlots	Cone colour	Cone size (ml) Mean*	SE	100 seed weight (mg) Mean*	SE	Filled seeds per cone Mean*	SE
I	183	- light brown cones with no grey colour present	5.6^{bcd}	0.1	312.2^{c}	4.3	18.1^{a}	0.5
II	189	- light brown cones with only slight greyish tinge on the weathered or exposed side	5.7^{abcd}	0.1	360.3^{a}	5.3	16.2^{b}	0.3
III	197	- cones with up to 50% greyish colour	6.1^{a}	0.1	334.2^{b}	5.1	16.7^{b}	0.4
IV	191	- cones with more than 50% greyish colour, but with some brown still present	5.8^{abc}	0.1	315.3^{c}	4.2	15.8^{b}	0.4
V	182	- cones totally grey with some black colouration present	5.3^{e}	0.1	313.0^{c}	5.2	13.0^{c}	0.4
VI	196	- black cones which may be covered with lichens	5.9^{ab}	0.1	319.3^{c}	4.0	11.8^{d}	0.3

*Within columns, means followed by the same letter are not significantly different at 5% level (Duncan's multiple range test).

Germination

Germination began on the fourth day and by day eight 95% of the germination was complete. Germinative capacity averaged 75%. Practically all ungerminated seed was dead or rancid; less than 1% of the seed was found to be empty.

Germinative capacity of jack pine seed generally decreased with increasing cone age (Table 2). Cone age class I had an average germinative capacity of 88%, while in class VI this had decreased to 55%. The highest germinative capacity in this study was found in one replicate of class I which had 100% germination, and the lowest occurred in a replicate of class VI which had 23% germination. However, germination results were more variable for the older cone age classes as indicated by the larger standard errors in Table 2.

Germinative energy also declined with increasing cone age as seeds from young cones germinated more rapidly than those from older cones (Figure 1). For example, by the sixth day the cumulative percent germination for class I averaged 84% but only 34% for class VI. Germination of seeds from cone age class V and VI was poorer and slower as indicated by Czabator's (1962) germination value (Table 2) when compared to seeds from class I and II which had prompt, uniform germination. The maximum germination value (22.44) was found in cone age class I whereas the minimum value (3.48) was found in class VI.

Tree-to-tree variation in germination results was statistically significant. The best tree had an overall germinative capacity of 90% (all cone age classes combined), while the poorest averaged 60%. In some trees even old cones retained seed of high viability while in other trees even the seeds from young cones had poor viability. The variation in viability between trees usually occurred throughout all cone age classes; if a tree showed poor germination in any given cone age class, it was likely to have poor germination in all classes.

Abnormal germinants increased with increasing cone age (Table 2). In cone age classes I to III, abnormal germinants averaged only 0.6% of all seeds, but in classes IV-VI abnormal germinants increased to an average of 3.7%. Cone age V contained both the maximum number of abnormal germinants, averaging 4.6% and the largest amount of variation.

Viable Seeds

The number of viable seeds (germinative capacity x yield of filled seeds) per cone exhibited a reduction with increasing age of cone (Figure 2). Cone age class I had twice the number of viable seeds per cone as cone age VI. When comparing filled and viable seeds per cone, the difference averaged only 2 to 3 seeds up to class IV (5-6 years), beyond which the difference increased to 4 to 5 seeds per cone.

Table 2. Effect of cone age upon germinative capacity, germination value, and abnormal germination (N=40).

Cone age class	Germinative capacity (%)		Germination value		Abnormal germinants (%)	
	Mean*	SE	Mean*	SE	Mean*	SE
I	87.8^{a}	1.1	19.1^{a}	0.3	0.4^{c}	0.1
II	85.8^{a}	1.4	17.6^{b}	0.4	0.6^{c}	0.1
III	80.3^{b}	1.2	16.1^{c}	0.3	0.9^{c}	0.1
IV	75.6^{c}	1.6	14.8^{d}	0.4	3.2^{b}	0.5
V	65.2^{d}	2.1	11.9^{e}	0.5	4.6^{a}	0.7
VI	55.4^{e}	2.5	9.4^{f}	0.5	3.4^{b}	0.6

*Within columns, means followed by the same letter are not significantly different at 5% level (Duncan's multiple range test).

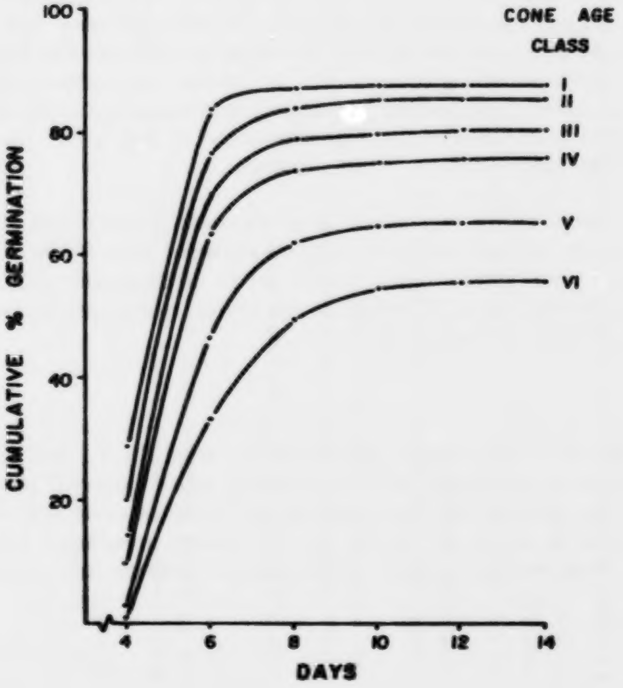

Figure 1. Cumulative percent germination of jack pine seed by cone age classes.

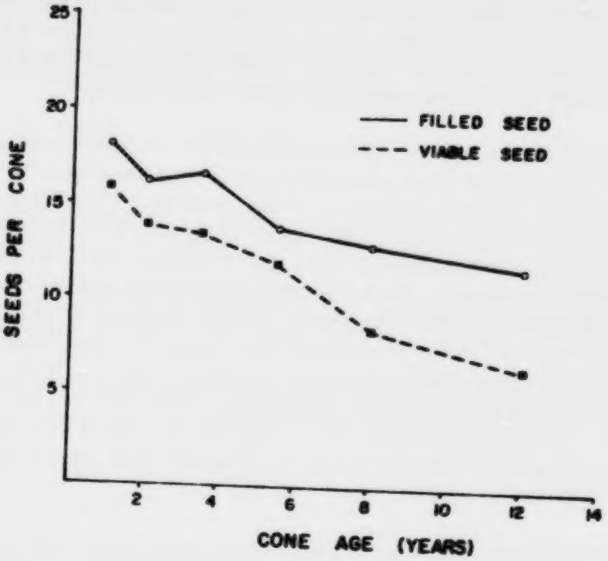

Figure 2. Comparison between filled and viable seeds per cone in relation to cone age. Cone age is plotted at the mid-point of the cone age class.

DISCUSSION

Increasing age of cone in jack pine resulted in both a decrease in seed yield and germination, as seeds from one year old cones averaged 15.9 viable seeds per cone and this decreased to 6.5 viable seeds in cones ten years and older. This phenomenon is in agreement with Roe (1963) who found cones eight years and older from 70 to 80 year old jack pine stands yielded less than half the seeds, both total and viable, than young cones. In lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) a closely related species to jack pine, Wang (1978, 1979) found both seed yield and germinability not affected by cone age (up to 15 years old). Thus it appears that this phenomenon does not apply to all northern temperate pines.

The reason for the decrease in filled seeds in unopened cones is unclear, although it may be the result of opening and closing of older cones as suggested by Roe (1963), poor seed years, or sampling older cones which in effect may be remnant cones and not representative of the original population. Presently it is impossible to detect if older cones on standing trees have opened and then closed. Old cones have the same morphological appearance as young cones, although they are darker in colour and somewhat softer. Scales from the infertile, basal region of the cone are generally the first to flex away from the cone axis. Beaufait (1959) suggested that if old cones were to open, it would be unlikely that they could return to their original form, as excised scales failed to return to their original position after passing through a desiccating series of wetting and drying.

Another possible explanation for this reduction in seed yield is that older cones could have been taken selectively by squirrels at the time of their maturity thereby affecting the uniformity of crop during the sample year. Large cones may be preferred by squirrels and it is these cones which would normally have a higher seed yield. In addition, older cones which were produced at an early age in the tree's life history but sampled at a later date, may have a poor seed yield because they were produced when the trees were young.

There have been contradictions in results between authors with regard to the influence of age of jack pine seed upon germinative capacity and energy (Schantz-Hansen 1941; Eyre and LeBarron 1944; Noakes 1946; Rudolph and Ralston 1953; Beaufait 1959; Roe 1963; Nikoncuk 1969; Ahlgren 1970; Popovich *et al.* 1970). This study clearly demonstrated that germination, both in terms of germinative capacity and germinative energy, declined with increased age of cone, while the incidence of abnormal germinants increased. These aspects of natural seed storage are specific consequences of seed deterioration (Delouche and Baskin 1973). Seed deterioration is a poorly understood process, but the evidence indicates that seed deterioration is controlled by both environmental (moisture and temperature) and genetic factors. Internal changes which chemically alter the food reserves or change the gene structure have been cited as either the cause or effect of seed deterioration during aging (Kozlowski 1972).

The net result of this decrease in filled seed yield and germination was a significant reduction in viable seeds, especially after cone age IV. Cone age IV (5-6 years) contained 30% more viable seeds per cone than cone age V (7-9 years). This pattern suggests that only cones up to 6 years of age should be collected. Cones six years and younger can be recognized by their partial brown colouration. Cones that are totally grey, black or lichen covered should be avoided.

Seeds from cones up to six years of age are especially desirable for nursery and container stock production, where both a high germinative capacity and energy are required. This, in turn, will result in an even and rapid rate of seed germination, and seedling stock of a more uniform size.

Seeds from all-age collections might be acceptable for direct seeding especially when cones are mechanically harvested. In mechanized collection systems, the cost of removing the older cones would not be justifiable. Owing to the lower germinative capacity, a greater number of filled seeds from all-age collections would have to be applied to achieve a similar sowing rate of viable seeds than if only seed from cones 1-6 years were used. Delayed germination from the lower germinative energy of older cones may result in the continued appearance of new germinants some time after seeding.

SUMMARY

It is recommended that only cones up to six years of age be collected for nursery and container stock production. These are cones that have some brown colouration present and are not entirely grey. This will maximize cone collection efficiency and improve upon seedling size uniformity since cones up to six years of age had both a high filled seed yield and a high germinative capacity and energy. Cones from all-age collections are acceptable for direct seeding where cones are mechanically harvested. However, the sowing rates should be much higher when using all-age collections due to their lower germinative capacity.

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THE RETENTION OF SEED VIABILITY IN SEMI-SEROTINOUS BLACK SPRUCE CONES

by V.F. Haavisto

Great Lakes Forest Research Centre
Canadian Forestry Service
Sault Ste. Marie, Ont.
P6A 5M7

ABSTRACT

In a series of studies, semi-serotinous cones of black spruce, from a variety of sites, were collected and aged to determine the effect of natural seed storage on seed yield and seed quality. Trees ranged in age from 18 to 140 years. The results indicate that seed viability is retained for several years following cone maturity. The quantity of seed per cone decreased with increasing cone age.

RÉSUMÉ

Dans une série d'études, on a prélevé et vieilli des cônes semisérotinaux d'épinette noire de diverses localités, afin de déterminer l'effet du stock naturel des graines sur leur comportement et leur qualité. L'âge des arbres variait de 18 à 140 ans. Les résultats indiquent que la viabilité des graines se maintient plusieurs années après la meturation des cônes et que le nombre de graines par cône diminue avec l'âge de celui-ci.

INTRODUCTION

Black spruce (*Picea mariana* (Mill.) B.S.P.) is a North American species with wide ecological amplitude. It is commonly found in pure stands on a variety of peatland sites, and in mixed stands on upland mineral soil sites. The species is in demand for the pulp and paper industry.

Black spruce have cones which retain seed on the tree for some years following cone ripening (Vincent 1965). With the semi-serotinous nature of the cones, it is imperative to understand the trends in seed viability as they relate to cone age. This natural storage on trees can be of considerable importance, not only for purposes of seed availability for nursery production, but also for natural regeneration systems.

This paper summarizes the results from a series of seed quantity and quality studies in black spruce, carried out over the past 14 years. The potential for using seed from older cones is evaluated. For the sake of completeness, the early studies in this series, and previously reported upon by Vincent (1966) and Haavisto (1975), are briefly summarized.

MATERIALS AND METHODS

In the series of studies conducted between 1965 and 1976, trees were chosen randomly from stands occurring on a variety of site types in northeastern Ontario. The stands ranged in age from 18 to 140 years. The older, larger trees from natural stands were felled using ropes and pulleys to lower the trees to the ground, thereby minimizing loss of cone on impact. Cone-bearing tops of the younger, shorter trees, from both natural stands and a plantation were removed using a short ladder.

All cones were collected and aged. Since cones of northern hemisphere conifers always form on the previous year's twig, each cone was readily aged using a combination of internode counts and counts of annual rings on the cross-section of the twigs. Cones originating in different years were kept separate throughout all phases of the investigations. The seeds were extracted by using 3 cycles of:

1) soaking in lukewarm water for 2 hours,
2) drying at 60°C for 24 hours, and
3) tumbling (for 30 minutes) in a screened drum designed specifically to handle small lots of cones.

The resulting seed was not cleaned. Investigations not reported herein have indicated that more than 90% of all seed is removed from the cones using the aforementioned methodology.

Germination tests were conducted using duplicate 100-seed samples (unclean) placed on moistened germination paper over a layer of absorbent material (cellulose wadding) in closed petri dishes. Germination conditions were 27°C for 16 hours with light, alternating with 8 hours of darkness at 20°C. Germination was checked once every 7 days and all germinates (radicle greater than 3 mm long) were removed. All ungerminated seeds were cut at the end of the 28 day test to ascertain whether they were filled or empty.

The percentage of the seeds that germinated (percent viability) and the ratio of seeds that germinated as compared to the total of the filled seed in the test were calculated.

RESULTS

In the first experiment, conducted by Vincent (1966) in northeastern Ontario, the per-cone seed yield from overmature (140+ years) peatland black spruce decreased markedly by the fifth year (Table 1).

Table 1. Seed quantity and quality from cones of different ages on 5 overmature black spruce trees collected in September 1965. (From Vincent 1966).

Year Cone Formed	Total Seed/Cone	Filled Seed/Cone	% Viability
1965	24.2	7.7	76.3
1964	20.0	5.7	47.5
1963	16.1	4.2	49.1
1962	8.1	2.1	43.9
1961	6.7	1.2	58.6

A study conducted between 1965 and 1968 consisted of annually harvesting all cones from groups of 5 trees during 3 consecutive years (Haavisto 1975). The trees ranging in age from 120 to 140 years were from typical productive lowland black spruce sites. The results (Table 2), indicated that both seed yield and seed viability decreased with increased age of cones. By the end of the 7th growing season, only about 25% of all seeds remained in the cones.

Table 2. Seed yield (all seeds) and viability from cones of different ages, based on averages of 15 overmature peatland black spruce trees collected between 1965 and 1967 in northeastern Ontario.

CONE YEAR	SEED/CONE	% VIABILITY
1967	13.7	57
1966	12.0	56
1965	12.3	60
1964	8.0	52
1963	6.7	44
1962	4.3	30
1961	3.7	30

There was little difference in the viability of the seed during the first 3 to 4 years. Thereafter, the viability decreased markedly. Some viable seed was obtained from cones over 20 years old.

In another overmature (120 years) peatland stand (Case Twp. northeastern Ontario), all cones were collected from 5 trees in 1974. Results (Table 3) indicate that viability was considerably higher for the 2nd and 3rd years than for the first (current) year. There was a decrease in viability in the 4th year. Similar trends held for the ratio of germinated seeds to total filled seeds.

Table 3. **Average percent viability and the ratio of germinated seeds to total filled seeds from one- to four-year-old cones on a 120-year-old black spruce tree (northeastern Ontario) collected in 1974.**

CONE YEAR	% VIABILITY	RATIO OF GERMINATED SEEDS TO TOTAL FILLED SEEDS
1974	40.1	.889
1973	59.5	.940
1972	60.9	.955
1971	37.0	.799

Five trees were cut from an exceptional black spruce stand (65 years) growing on a fresh upland site in northern Ontario. Only cones of the three most recent years were found. The results (Table 4) indicate that a dramatic decrease in seed yield was found by the third year. Seed viability remained more-or-less the same. The ratio of germinated seeds to total filled seeds remained very high for the 3 years, and almost all seed with a potential for germinating did so.

Table 4. **Average seed yield, percent viability and the ratio of germinated seeds to total filled seeds for one-to-three-year-old cones from five 65-year-old black spruce trees in northern Ontario.**

CONE YEAR	SEEDS/CONE	% VIABILITY	RATIO OF GERMINATED SEEDS TO TOTAL FILLED SEEDS
1976	48.9	69.5	.987
1975	18.6	70.6	.978
1974	5.7	63.1	1.000

In the young plantation (trees 18 years old) from northwestern Ontario, seed yield decreased very rapidly after the first year (Table 5). Seed viability, however, remained high during this period.

Table 5. **Average quantity and quality of seeds from cones of five 18-year-old black spruce trees in an upland plantation (northwestern Ontario).**

CONE YEAR	SEED/CONE	% VIABILITY
1976	22.1	50.5
1975	10.1	42.5
1974	7.5	46.5
1973	2.4	14.0
1972	1.5	17.0
1971	1.8	0.0

DISCUSSION

All of the results presented herein have shown that seed viability is retained for several years following cone maturity in black spruce. Considerable seed can be lost from the cones over a period of time.

Comparative results are not available for assessing seed retention in cones from trees of different ages growing on similar sites. The results presented herein suggest that the seed yield per cone is higher in younger trees; however, retention is poorer. The 65-year-old stand of exceptional trees at Fraserdale, Ontario (Table 4) was situated on a much better-drained site than the trees sampled in Kennedy Twp. (Table 2) and Case Twp. (Table 3).

Black spruce cones in stands situated on peatlands (Tables 2 and 3) seemed to retain seed better than those on the drier mineral soil upland (Tables 4 and 5). This may be a reflection of physiological differences, e.g., under wetter conditions the trees retain more moisture, and consequently there is a higher moisture content in the cones. Seed quantities were invariably higher in the first year's crop on upland sites.

Seed viability does not change appreciably during the first few years, and it is therefore suggested that as long as good seed is retained, cones of several ages can be collected (Haavisto 1979). If this is true, mechanized cone collection would be appropriate.

The 65-year-old exceptional stand sampled at Fraserdale yielded the highest numbers of seeds per cone. Forest Managers should therefore locate and reserve such stands for seed collection purposes.

Some trees from the 14-year-old plantation yielded good cone crops whereas others did not. The average values presented in Table 5 suggests that young plantations might be very good sources of seed. However, studies are necessary to ascertain whether seed quality from young stands is acceptable, and to elucidate reasons for between-tree differences.

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SUMMARY REPORT

Technical Session 2: The effects of cone storage conditions on seeds

Chairperson: D.G.W. Edwards
Co-chairperson: Y. Lamontagne

Five papers were presented relating to the effects of seed retention in the cones on quality. All the authors were North American which may reflect a difference in concern from that of seed workers in other countries.

Retention of seeds in the cone may be due to either a mechanism developed within the species for naturally retarding seed dissemination, or the delay in seed removal after cone harvest. Both aspects were addressed.

Leadem concluded that seed quality in five conifers was largely unaffected by up to 6 months' storage in the cones. No advantage was gained in using refrigerated storage facilities, and outdoor, open-air storage promoted the desired gradual drying of the cones. Cones that were in poor condition through poor field handling did not store well, indicating that greater efforts should be made to ensure that cones are not shipped to the processing plant in a moist condition. The need for prolonged seed storage in the cones arises frequently because the processing plant can handle only limited quantities at any one time. In the discussion period it was mentioned that other countries have also encountered the requirement for storing large volumes of cones for prolonged periods, and that good results have been obtained. Caution was voiced over storage of moist cones outdoors when low temperatures may cause frost damage, and certain species may require indoor storage for this reason. In other species, frozen cones are easier to work with since the natural cone resins have solidified. Leadem's study related to storage in burlap sacks and the retention of moisture by the innermost cones was believed to be problematical even though there was good ventilation around the sacks. This method of storage may be less suitable when the initial condition of the cones is poor; storage in trays being a better alternative.

Freezing damage to *Abies procera* seeds was reported by Tanaka who attempted to quantify the effects by predicting the critical temperatures at which losses in germinability occur. Reducing cone moisture content was discussed as a means of minimizing such damage when cones must be stored outdoors. Heating the storage facilities, when possible, was suggested as an alternative and this has been found elsewhere to minimize mould damage. Again, the requirement of good ventilation during storage was emphasized.

Haddon and Winston found viability of seeds from artificially ripened *Picea glauca* cones remained high after 2 years of seed storage. Cone storage at 5°C appeared to eliminate seed dormancy (as measured by the prechill requirement) in most seedlots despite the 2-year storage period. During the discussion it was observed that cone opening in other species was more difficult following artificial ripening and the reduced seed yield offset the benefits of high viability. The requirement to prechill *Picea glauca* seeds was questioned and the authors responded that they had found more rapid germination when white spruce seed had been pretreated, although this factor was not examined in their study. Other literature reports have shown that prechilling can be detrimental to white spruce seed quality suggesting that further study of this phenomena is required. Published seed manuals, such as those concerning U.S. species, are not considered adequate for Canadian trees and it was suggested that a comprehensive treatise be compiled for this country. A question was raised whether seed maturity could be related to the cessation of vegetative growth in the parent tree. Other studies have reported some correlation between seed maturation and accumulated heat sums, but there is little evidence to reliably support the use of such indices.

Baker found both seed yields and seed quality decreased with increased age of *Pinus banksiana* cones. Although cones older than 6 years yielded germinable seeds, germinants were less vigorous. It was recommended that collection in this species be confined, as far as practical, to cones younger than 6 years, cone coloration being a good method of assessing cone age in the field. Older cones, which cannot be rejected in mechanical cone harvesting, are acceptable for providing seeds for aerial seeding use, but reduced extractability lowers their desirability. As the discussion showed, seed extractability remains a poorly understood phenomenon and the potential for seed yield can only be obtained by complete dissection of the cones. It was pointed out that while seed germinability apparently decreased with cone age in Baker's study, no attempt had been made to discern whether this reduction was actually due to deterioration or to dormancy. Seeds of this species are not normally dormant so the effects of prechilling were not covered.

Haavisto reported that due to the semi-serotinous cone habit in *Picea mariana*, seeds may be retained on the tree for extended periods. From several years' studies, viability had been found to diminish rapidly in 2-year-old cones, stabilize for a further 2 to 5 years, then rapidly decrease again, although viable seeds have been found in cones more than 20 years old. The silvicultural implication of these results were discussed. Haavisto stressed that all his germination data was based on uncleaned seeds, that is, empty seeds had not been removed, primarily to avoid processing damage. During the discussion it was pointed out that the varying levels of empty seeds may have been related to the observed decreases in quality with increasing cone age. Variation in seed quality from year to year may also have an influence. Crown exposure affects the amount of cones produced and the author explained that intermediate trees, with crowns in some degree of shade, produced more cones and gave higher yields of more viable seeds.

TECHNICAL SESSION 3: EFFECT OF INSECTS AND DISEASES ON CONE AND SEED STORAGE

Chairperson: W.H. Fogal
Co-chairperson: D. Chai

SPRUCE CONE BEETLE (ANOBIIDAE: *ERNOBIUS* SP.) REDUCES THE NATURAL STORAGE OF SEED BY BLACK SPRUCE (*PICEA MARIANA*).

Hugh O. Schooley

Canadian Forestry Service
Newfoundland Forest Research Centre
St. John's, Newfoundland

ABSTRACT

In Newfoundland an unidentified beetle (*Ernobius* sp. (Anobiidae)) destroys the inventory of seed normally retained in old black spruce (*Picea mariana* B.S.P.) cones that persist on the trees for many years. Insufficient seed may remain to re-establish stands following harvest or destruction by fire. Substantial seed losses to this beetle can also occur in cones stored prior to the extraction of seed. This cone beetle is known only from Newfoundland. Other locations throughout the range of black spruce should be examined for the pest.

RÉSUMÉ

A Terre-Neuve, une espèce non identifiée du genre *Ernobius* (Anobiidae) détruit les réserves de graines d'épinette noire (*Picea mariana* B.S.P.) normalement conservées dans les cônes âgés qui restent sur les arbres pendant de nombreuses années. Il y a risque qu'il ne subsiste un nombre suffisant de graines pour la reconstitution des peuplements après leur exploitation ou leur destruction par le feu. L'insecte peut également causer des pertes importantes dans les cônes stockés avant l'extraction des graines. Sa présence n'est connue qu'à Terre-Neuve mais devrait être recherchée à d'autres endroits dans l'aire de l'épinette noire.

INTRODUCTION

Several species of *Ernobius*, members of the death-watch beetle family (Anobiidae), damage the cones of various conifers (Keen 1958; Stadnitskii *et al.* 1978). Most species feed only in mature or dead cones and so are not important in seed production. However, one European species, *E. abietis* Fabr. will occasionally attack female flowers of Norway spruce, *Picea abies* (L.) Karst. Larval feeding at the base of young cones may cause them to drop prematurely from the trees (Wiersma 1978). Larvae of another European species, *E. tabidus* Kew. frequently occur in the strobiles of spruce which have already been damaged by lepidopterous pests. The damage of both these species is considered insignificant because it affects only a few cones (Stadnitskii *et al.* 1978).

An undescribed *Ernobius* sp. has been causing significant damage to black spruce, *Picea mariana* B.S.P., cones in Newfoundland. The beetle feeds on the interior of old cones that characteristically persist on trees for many years. These old cones usually retain some of the seed they have produced and this seed reservoir allows the natural re-establishment of black spruce stands following harvest or destruction by fire (Vincent 1965). Seed losses caused by beetle feeding may result in insufficient regeneration for future stands. The beetle also destroys many seeds in cones held in storage prior to seed extraction.

In this paper, I will give some general information about the spruce-cone beetle, its distribution in Newfoundland and the amount of damage we have experienced.

METHODS AND MATERIALS

Description of the seasonal history of the cone beetle, *Ernobius* sp., its life stages and its damage were made from field observations and notes made while dissecting cones.

The distribution of the beetle and estimates of the percent of cones damaged are based on cone samples collected in 1978 from various crop years on individual trees. When the crop was 10 or fewer cones then all cones were collected as a sample. Where there were more than 10 cones on a tree then 10 cones plus 10% of the visually estimated number of cones were collected. Cone crop size was based on the following criteria:

Crop Size	Cone per tree by years
Light (L)	50 or fewer (14 or fewer cones per sample)
Medium (M)	60 to 150 (15 to 24 cones per sample)
Heavy (H)	More than 160 (25 or more cones per sample)

In the late summer of 1977 a large volume of cones that had been produced in 1976 were collected from a young black spruce stand located near Gambo in central Newfoundland. These cones were exposed to cone beetle infestation during the whole summer of 1977. Under normal weather conditions the majority of seed in these cones would have been released during the late fall and winter of 1976 and the spring of 1977. However, poor weather conditions had prevented this release and the cones contained their full complement of seed when collected. The cones were stored, prior to extraction, in sacks loosely piled in an unheated, weather-tight building. The extraction of seed from those cones was completed by late summer of 1980.

The stored cones were sampled at various dates between July 1978 and September 1980. On each sampling date unbiased collections of about 130 cones were taken from each of 4 to 8 sacks located in the interior of the storage pile. Each of the cones was examined for holes through which the adult beetles emerge. Holed cones were bisected longitudinally to classify damage into four categories; - 1/4, 1/4 to 1/2, 1/2 to 3/4 and 3/4 of the exposed seed bearing area of the cones destroyed. Cones that were not holed by emerging adults were also bisected to estimate damage and then they were dissected to determine the presence of immature stages of the insect.

RESULTS AND DISCUSSION

Biology of the Beetle

Flight activity and oviposition of the medium to dark brown, 3 to 5 mm long beetles begin in mid-July, peaks at the end of July and probably continues throughout the remainder of the summer. White, circular eggs, 0.2 - 0.3 mm in diameter were usually deposited between the scales near the base of mature cones produced during the previous year's growing season. Larvae are white with distinct legs, light brown heads and long body hairs. During the summer larvae excavate irregularly shaped feeding cavities within the axes and seed bearing areas of the cones. Normally only one larva develops within each cone, however, two larvae per cone were occasionally observed. White, naked pupae may develop before the onset of winter but spring pupation appears more common. Pupation occurs in the feeding cavity near the base of the cone and adults emerge through round holes cut in the surface of the cones near the pupation sites.

Occurrence of the Beetle and its Damage in the Forest

Adult beetles and larvae have been collected at several locations; however, the distribution of the beetle is also being revealed by examining cones for adult emergence holes. This evidence of damage has been found at all 31 locations where collections have been made and indicates that the beetle is present throughout the Island (Fig. 1). Cones produced as early as 1958 were examined but the oldest cones with exit holes were produced in 1963.

As many as 50% of cones in individual collections were damaged; however, average damage of all samples in any given year did not exceed 18% (Fig. 2). At one intensively sampled location the size of cone crops was compared to the percentage of cones damaged each year (Fig. 3). No relationships were apparent. A gradual decrease in beetle population levels between 1963 and the present was indicated by a least square trend of the average annual percent of cones with beetle damage (Fig. 2). The decrease in population levels may be greater than indicated by this trend line if beetle damaged cones deteriorate and drop from the trees before undamaged cones produced during the same crop year.

In Newfoundland, cones retain about one-tenth of their seed after 13 or more years (Schooley *et al.* 1979). This seed reservoir becomes available to re-establish stands following harvest or forest fire (Wilton 1963). In recent years cone production in many black spruce stands in Newfoundland has been virtually eliminated by damage caused by the spruce budworm (Schooley 1980). This failure

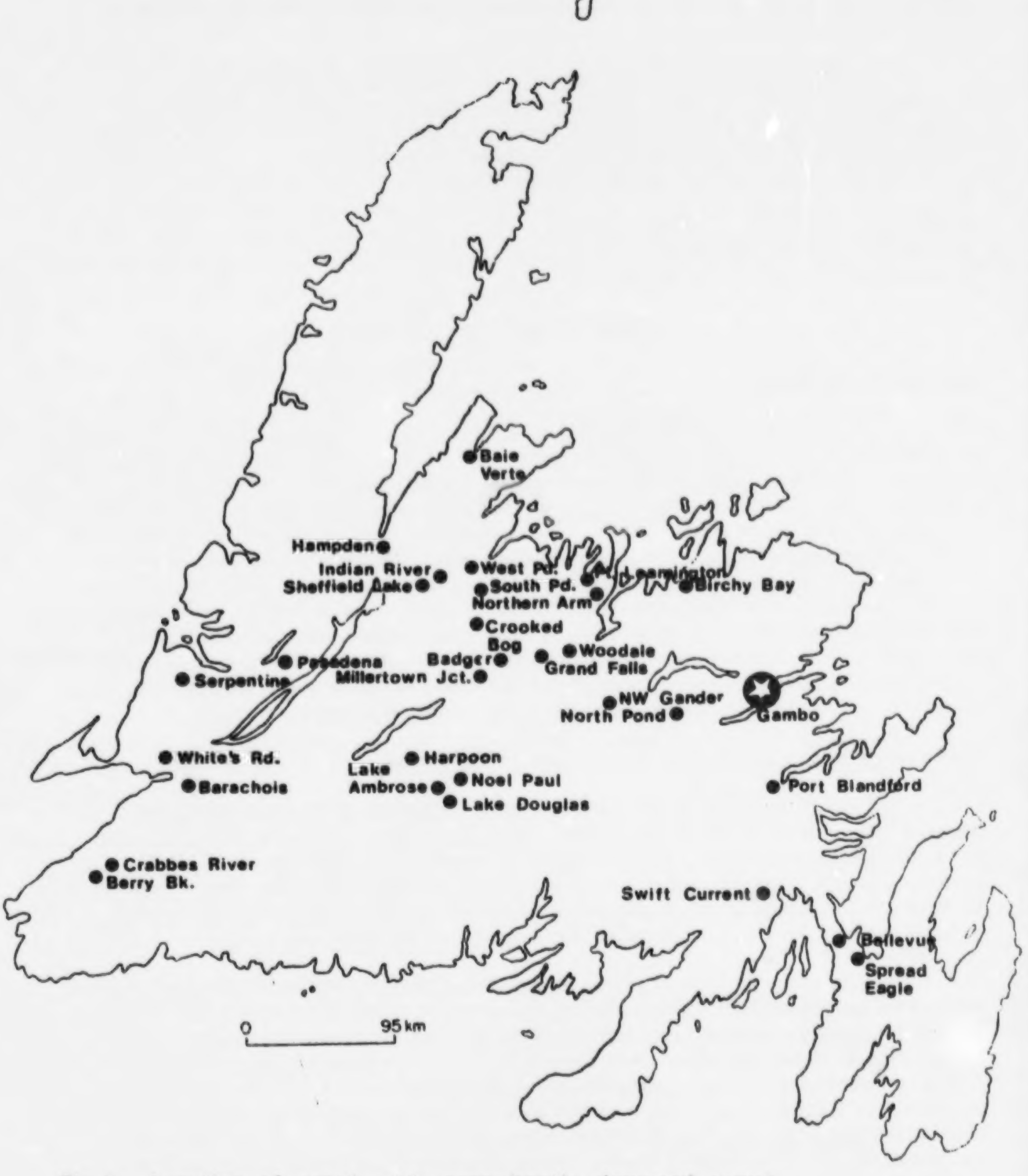
Baie Verte
Hampden
Indian River
Sheffield Lake
West Pd.
South Pd.
Northern Arm
Pt. Leamington
Birchy Bay
Crooked Bog
Woodale
Grand Falls
Badger
Pasadena
Serpentine
Millertown Jct.
NW Gander
North Pond
Gambo
White's Rd.
Barachois
Harpoon
Lake Ambrose
Noel Paul
Lake Douglas
Port Blandford
Crabbes River
Berry Bk.
Swift Current
Bellevue
Spread Eagle
0
95 km

Fig. 1; Location of areas with cone-beetle damaged cones.

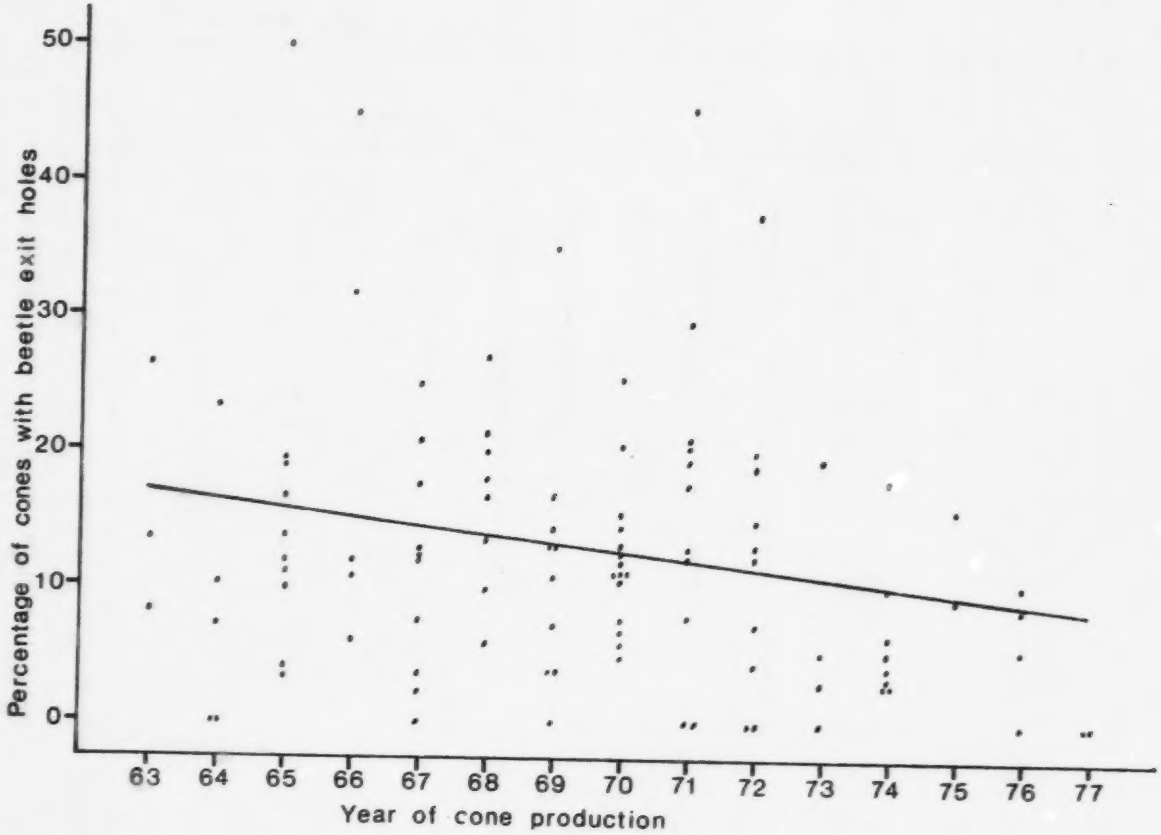

Fig.2; Percentage of cones collected with beetle exit holes in various years from 1963 to 1977.

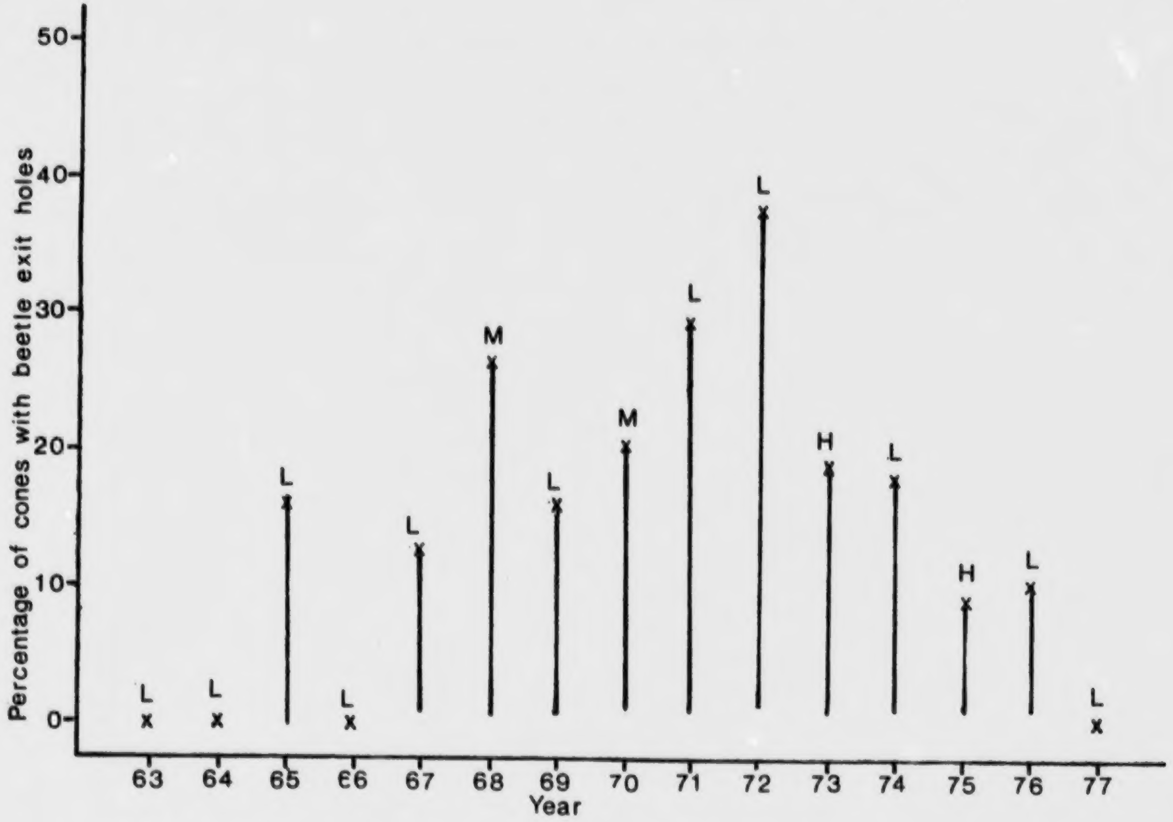

Fig.3 Relationship of percentage of cones damaged and cone crop size in various years from 1963 to 1977.

to produce cones has greatly increased the importance of the seed reservoir in old cones. The occurrence of the cone beetle on black spruce has only been reported in Newfoundland. Other locations throughout the geographic range of black spruce should be examined for the pest.

Beetle Damage During Cone Storage

Under normal circumstances it is unnecessary to store cones for a long period prior to extraction. However, when Newfoundland's seed extracting facility was dismantled and construction of a new extraction plant was delayed cone storage became necessary. A large volume of black spruce cones collected in September 1977 were stored until the summer of 1980. A large number of cone beetle adults were observed emerging from the cones in July, 1978. About 6% of cones sampled at that time had adult emergence holes (Table 1). The percentage of cones with emergence holes increased with time to about 26% of the cones by the end of the 1980 growing season. Damaged cones without emergence holes increased total damage to nearly 24% of the cones by the end of the first year of storage and to about 34% by September 1980 after three years storage.

Table 1. Number of stored cones examined, percentage of these with adult emergence holes and total percentage of cones damaged by cone beetles on various dates between 1978 and 1980.

Date	No. cones examined	% cones holed	Total % cones damaged
July 1978	545	5.7	22.4
Sept. 1978	638	11.1	-[1]
Nov. 1978	1011	14.1	23.8
Mar. 1979	1183	12.0	-[1]
July 1979	943	19.9	24.7
Feb. 1980	736	18.6	26.1
Sept. 1980	840	25.8	33.9

[1] No cone dissections were made on these dates.

The cones were infested by beetles during the whole storage period. Between July and August 1978 there was a sharp drop in the beetle population because many adults of the first generation to develop in the cones dispersed away from the storage shed. However, as indicated by the annual increase in the percentage of cones damaged, the infestation continued at about one-third of its initial level (Table 1). Living larvae and adults were present in the cones examined in September 1980 and damage would have continued to increase had the cones not been processed.

More than 3/4 of the seed bearing parts of cones were destroyed in about 63% of the cones from which adult beetles had emerged (Table 2). The remaining cones with adult emergence holes were about equally divided among the three classes with lesser amounts of damage. Many of these cones had small amounts of damage. They had been infested by larvae moving from one cone to another or by adults boring from one cone to another while attempting to emerge from the densely packed sacks of cones.

The amount of seed extracted from beetle damaged stored cones was about one-half that obtained from cones that were collected at a later date and not stored (Table 3). The additional transport and handling required by storage probably resulted in a small amount of seed being lost. However, most of the difference in seed yield must be attributed to the cone beetle infestation. As already indicated about 34% of the cones had been infested and in most infested cones more than 3/4 of seed bearing parts of the cones had been destroyed. The cone beetle also caused much higher than normal seed losses during seed extraction. Damaged cones broke up when processed. This debris together with a large amount of insect boring dust and frass made recovery of seed very difficult.

Table 2. The amount of damage to the seed bearing parts of cones from which adult beetles emerged.

Amount of cone destroyed	Percentage of cones
1/4	11.5
1/4 to 1/2	13.0
1/2 to 3/4	12.1
3/4	63.4

Table 3. Weight of seed extracted from stored and unstored cones.

Collection location	Year collected	Weight of cones (kg)	Weight of seed extracted	
			Total (kg)	Per kilogram of cones (g)
Stored cones				
Gambo	1977	6408	39.8	6
Unstored cones				
Cormack	1979	2700	35.9	13
Victoria	1979	1995	22.1	12

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SPERMOPLANE MICROFLORA OF STORED SEEDS OF *TECTONA GRANDIS*, *BOMBAX CEIBA* AND *EUCALYPTUS* SPP. IN RELATION TO GERMINABILITY

J.K. Sharma and C. Mohanan
Division of Forest Pathology
Kerala Forest Research Institute, Peechi, Kerala, India.

ABSTRACT

Stored seeds of *Tectona grandis* Linn. f. (teak), *Bombax ceiba* Linn. *(Bombax)*, *Eucalyptus citriodora* Hook, *E. globulus* Labill., *E. grandis* Hill ex Maiden. harboured a rich spermoplane microflora. However, in *E. tereticornis* micro-organisms occurred in low frequencies. *Aspergillus* was the most predominant genus in all tree species, except *Bombax* where actinomycetes were the most frequent organisms. Actinomycetes, and 8 fungal genera, viz. *Fusicoccum*, *Gliocephalotrichum*, *Gonatobotryum*, *Humicola*, *Pestalotia*, *Pithomyces*, *Thielavia* and *Torula* are reported for the first time on seeds.

In addition to common saprophytic storage micro-organisms, the spermoplane microflora was comprised of numerous potential pathogenic organisms. These pathogens, which may be seed-borne in nature, are known to cause infection of seedlings and young plants of many agricultural crops.

In the blotter method, except for *E. tereticornis*, seeds of other tree species showed low germination; and this was directly related to percentage of seeds colonised by micro-organisms and frequency of storage microflora. A good percentage of healthy seedlings of teak, *Bombax*, *E. citriodora* and *E. globulus* were found to be attacked to various degrees by spermoplane organisms.

Poor germination of teak seeds and incidence of seedling mortality was comparable in blotter and growing-on methods. Mortality of seedlings was caused by *Fusarium*, *Alternaria*, *Curvularia* and bacteria.

RÉSUMÉ

Les graines stockées de *Tectona grandis* L.T. (teck), de *Bombax ceiba* L. (fromager), d'*Eucalyptus citriodora* Hook, d'*E. globulus* Labill. et d'*E. grandis* Hill ex Maiden abritaient une riche spermoflore. Chez *E. tereticornis*, très peu de micro-organismes étaient présents à de faibles fréquences. *Aspergillus* était le genre le plus fréquent chez toutes les essences sauf chez *Bombax* où les actinomycètes étaient les plus fréquents. Il s'agit de la première observation d'actinomycètes et de huit genres de champignons, *Fusicoccum*, *Gliocephalotrichum*, *Gonatobotryum*, *Humicola*, *Pestalotia*, *Pithomyces*, *Thielavia* et *Torula*, sur des graines.

Outre les saprophytes présents de façon courante sur les graines stockées, la spermoflore se composait de nombreux organismes potentiellement pathogènes. Ceux-ci, qui peuvent être naturellement présents sur les graines, sont des agents infectieux connus des semis et des jeunes plants dans de nombreuses cultures.

Avec la méthode du papier buvard, la germination des graines de toutes les essences sauf *E. tereticornis* a été faible et directement reliée au pourcentage de graines colonisées par des micro-organismes et à la fréquence de la spermoflore de stockage. Un pourcentage respectable de semis sains de teck, de *Bombax*, d'*E. citriodora* et d'*E. globulus* ont été attaqués à des degrés variés par la spermoflore.

La germination médiocre des graines de teck et la mortalité des semis étaient comparables pour les méthodes du buvard et à croissance poursuivie. La mortalité des semis était attribuable aux espèces *Fusarium*, *Alternaria*, *Curvularia* et à des bactéries.

INTRODUCTION

In India, seeds of forestry crops are required in large quantities every year to raise plantations. Seeds are generally collected from an old plantation and stored for some duration. Storage of seeds collected at various times of the year becomes essential since the nursery is raised taking into account the planting season.

Germinability depends much upon seed quality and storage conditions. Besides pre- and post-emergence seedling diseases, which may be seed-borne in nature, nursery stock is greatly affected due to germinability of seeds. ***T. grandis*** exhibits considerable variation in germination. Since teak fruits are directly sown, nursery stock obviously depends upon the germinability of seeds in fruit. The germination is usually found to be low (< 60 percent) (Troup 1921; Murty 1973; Ghosh 1977). The low germinability of teak fruits is largely attributed to emptiness, the percentage of which varies from area to area (Gupta and Kumar 1976). Although many studies have been conducted, the reason for low percentage germination is not fully understood. For a large scale planting programme, the low germinability of teak therefore continues to be a constant obstacle (Kamra 1974).

High percentage germination is reported for freshly collected seeds of ***Bombax***, which may drop to 20-30 percent in a year (Troup 1921). On the other hand, storage of eucalypts does not result in loss of germinability, if seeds are properly dried and stored at low temperature (FAO 1976). A satisfactory germination capacity can be maintained for a year or two in unsealed containers at room temperature. However, it has been experienced that seeds of some species, especially ***E. deglupta*** lose germinability fairly quickly, or even freshly collected seeds have low viability.

For the causes of poor germination, so far studies have been directed at seed dormancy, maturity and size, etc., but no attention has been given to deterioration of seed quality due to micro-organisms. Invasion of seeds in storage by micro-organisms has been well established from the extensive seed pathological work carried out on various agricultural and horticultural crops in the past twenty years (Christensen 1957; Christensen and Kaufmann 1965, 1969; and Neergaard 1977). These micro-organisms not only affect the quality of seeds by causing deterioration, decay, fat acidity, heating and musting, and toxin production but also the loss of germinability (Milner and Geddes 1946; Goodman and Christensen 1952; Christensen 1955). Seeds of forest tree species, which are equally rich in cellulose, fat, protein and carbohydrates are also liable to be affected by storage micro-organisms, especially fungi. Except for some reports on temperate pines (Grevatt 1931; Fisher 1941; Gibson 1957; Munjal and Sharma 1975), seeds of forestry crops of tropics, which are most vulnerable to biodeterioration due to climatic conditions, have not been studied. The present investigation is aimed at studying the micro-organisms associated with stored seeds of some economically important plantation species, ***Tectona grandis, Bombax ceiba, Eucalyptus citriodora, E. globulus, E. grandis*** and ***E. tereticornis*** and their probable role in affecting germinability, with special reference to teak.

MATERIAL AND METHODS

(i) Collection and storage of seeds:

Seeds of teak and eucalypts, supplied by the Geneticist, Tamilnadu Forest Department, Coimbatore, Tamilnadu were collected in 1979 and stored at room temperature (25-38°C) till May 1980. Thereafter seeds were stored at KFRI, Peechi. The seeds of different species were kept separately in loosely closed polythene bags and placed in plastic containers at room temperature (22-28°C) and R.H. (70-85 percent). At Coimbatore eucalypt seeds were treated with BHC 10 percent dust (Hexachloro cyclohexane) prior to storage to prevent insect infestation.

Seeds of *Bombax*, collected in January 1980, were supplied by the Genetics Division, KFRI and stored at room temperature in loosely capped plastic containers.

(ii) Extraction of teak seeds:

The seeds were extracted from the fruits using a modified nut cutter described by Dabral (1976), and the number of large and small well-filled seeds per fruit recorded. Seeds were stored in a sterile glass Petri dish at room temperature for two days before using in the experiment.

(iii) Dry examination of seeds:

One hundred seeds of each species were examined under a sterioscopic binocular microscope (50 x) to ascertain any discolouration and indications of fungal or insect infestation.

(iv) Incubation method - blotter method:

The standard blotter method for seed testing was employed (ISTA, 1966). A random sample of 200 seeds was taken for each of the species, except teak where the number of seeds was 242, extracted from 260 fruits.

The seeds were sown at equal distance in sterilized glass Petri dishes containing three moist filter paper disks (blotter). The number of seeds incubated per plate depended on the size of seed tested: 10 in the case of teak and *Bombax* and 20 for *Eucalyptus* spp. The plates were incubated in a growth chamber fitted with two cool white fluorescence tube lights at 30(±2)°C under 12 hours of alternating cycles of light and darkness. As the blotters became somewhat dry by the 4th or 5th day of incubation, a few drops of double glass distilled water were added. Observations on the presence of micro-organisms, germination of seeds and infection, if any, of young seedlings were recorded using the stereoscopic binocular microscope between 8 to 10 days after incubation. Identification of micro-organisms was attempted up to the generic level where possible. The data for more than one species of a genus were pooled.

(v) Growing-on method:

To assess the effect of storage micro-organisms, (some of which may also be seed-borne), on the germinability of teak seeds, a procedure of a growing-on method was followed. A total of 280 large (well-filled) seeds were extracted from teak fruits. Due to some practical problems, the seeds could not be sown immediately and had to be stored for 3 days. To prevent any fungal growth on the surface due to the prevailing high humidity (ca. 85-90 percent) a seed dressing of Bavistin 50 percent WP (2-(Methoxy-carbamoyl)-benzimidazole) was applied before storing in a plastic container.

The soil was steam sterilized (at 1.0 Kg/cm^2) for two hours. After cooling it was transferred into formaline treated (spray of 0.01 percent solution and left for 24 hours after covering them with polythene sheet) earthenwear pots. Seeds were sown in the pots the next day and watered frequently with double glass distilled water. Observations on healthy and diseased seedlings were recorded up to 30 days after the emergence. The affected parts from diseased seedlings were plated on PDA to isolate the pathogen.

RESULTS

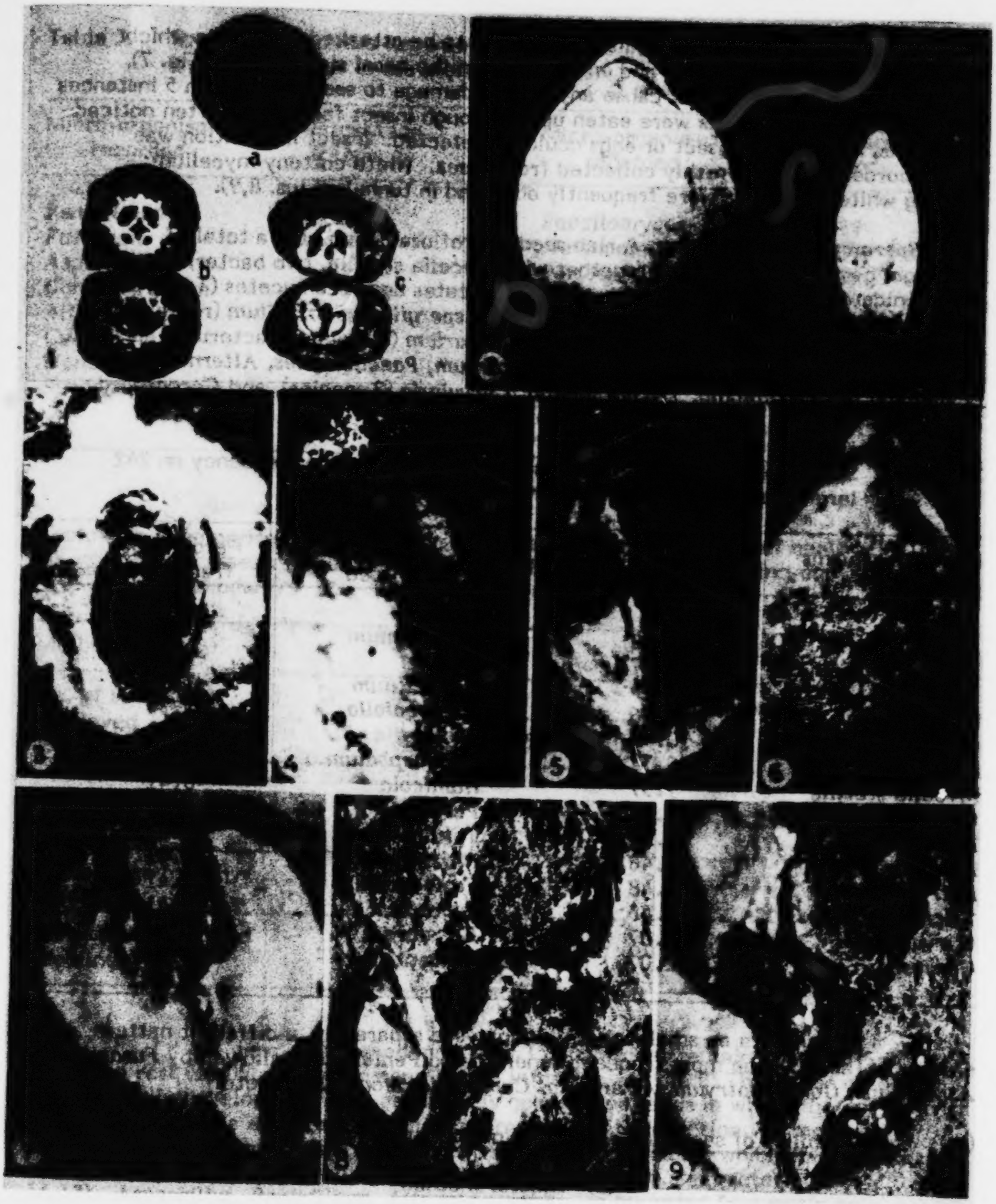
Teak

(i) Extraction of seeds - One hundred teak fruits yielded a total of 111 large and 29 small (well-filled) seeds (Table 1). All the fruits contained either one or two large and or small seeds, except in one fruit where 4 seeds (2 large and 2 small) were found (Figs. 1,2). At least one large seed was present in most of the fruits, however from 7 fruits only small seeds were extracted.

Table 1. Number of teak seeds extracted from 100 fruits

Number of seeds/fruit		Number of fruits	Total number of seeds extracted	
large	small		large	small
1	-	58	58	-
2	-	17	34	-
-	1	7	-	7
-	2	2	-	4
1	1	10	12	12
1	2	1	1	2
2	1	2	4	2
2	2	1	2	2
Total		100	111	29

(ii) Dry examination of seeds for fungal attack and insect infestation: Dry examination of seeds revealed that at least 18 percent of the extracted seeds were infected with fungi. *Penicillium, Aspergillus, Fusarium,* white and dark coloured mycelium and white ascocarps were most frequently encountered (Figs. 3 to 6). Seeds infected with *Aspergillus* and *Penicillium* were shrivelled and light in weight.



Figs. 1-9. ***Tectona grandis.*** 1, Fruit: a, basal view; b,T.S. showing 4 seeds (2 small and 2 large) in loculi; c, seeds (1 small, 1 large) in median V.S.; 2, large and small seed; 3, a seed completely deteriorated due to infection of ***Penicillium*** and ***Aspergillus***; 4-6, infection on freshly extracted seeds: 4, ***Aspergillus*** and ***Botryodiplodia***; 5, ***Fusarium***; 6, fungal colonies, unidentified; 7, insect tunnels bored from the basal side of fruit; 8, tunnel and seed colonised with fungus mycelium. Note ascocarps in the tunnel; 9, similar ascocarps growing on the seed.

A large number of fruits were found to be attacked by insects which made tunnels in the mesocarp and endocarp from the basal side of fruit (Fig. 7). Apparently the insect did not cause any visible damage to seeds, except in 5 instances (1.25 percent) where seeds were eaten up. Although insect faeces was often noticed in tunnels, no trace of insect or eggs could be detected. Insect infestation was also recorded in fruits freshly collected from trees. White cottony mycelium bearing white ascocarps were frequently observed in tunnels (Figs. 8,9).

(iii) *Spermoplane microflora:* A rich seed microflora comprising a total of 25 fungal genera was recorded together with mycelia sterilia, two bacteria and three unidentified genera belonging to hyphomycetes and ascomycetes (Table 2) (Figs. 10-15). The most frequent genera were ***Aspergillus*, *Penicillium*** (represented by respectively 6 and 4 species) followed by ***Fusarium*** (4 species) bacteria (2 species), ***Cephalosporium*, *Syncephalastrum*, *Gonatobotryum*, *Paecilomyces*, *Alternaria*** (2 species), ***Botryodiplodia*, *Trichothecium*, *Curvularia*** (2 species), and ***Cercospora***.

Table 2. Spermoplane micro-organisms and their percentage frequency on 242 large teak seeds in the blotter method.

Micro-organisms recorded	Percentage frequency	Micro-organisms recorded	Percentage frequency
Aspergillus	52.48	***Chaetomium***	1.65
Penicillium	52.07	***Mucor***	1.65
Fusarium	42.95	***Monocillium***	1.65
Bacteria	14.46	***Memnoniella***	1.24
Cephalosporium	10.36	***Pestalotia***	0.83
Syncephalastrum	5.37	***Oedocephalum***	0.83
Mycelia sterilia	5.37	***Humicola***	0.41
Gonatobotryum	3.72	***Periconia***	0.41
Paecilomyces	3.72	***Torula***	0.41
Alternaria	2.48	***Pithomyces***	0.41
Botryodiplodia	2.48	***Fusicoccum***	0.41
Trichothecium	2.48	***Sporothrix***	0.41
Curvularia	2.07	***Hansfordia***	0.41
Cercospora	2.07	Unidentified	26.03

When data on small seeds was analysed separately, a different pattern emerged (Table 3), the most frequent genera being ***Penicillium*** followed by ***Fusarium*, *Aspergillus*, *Gonatobotryum*, *Alternaria*, *Curvularia*, *Monocillium***, etc.

(iv) Germinability of seeds in relation to infestation -

(a) Blotter method

The overall germination performance of large seeds was very poor (25.39 percent) since many of them failed to germinate (Table 4). Ungerminated seeds were profusely colonised with ***Aspergillus*, *Penicillium*, *Fusarium*, *Cephalosporium*** and bacteria. Small seeds did not germinate at all (Figs. 10,11).

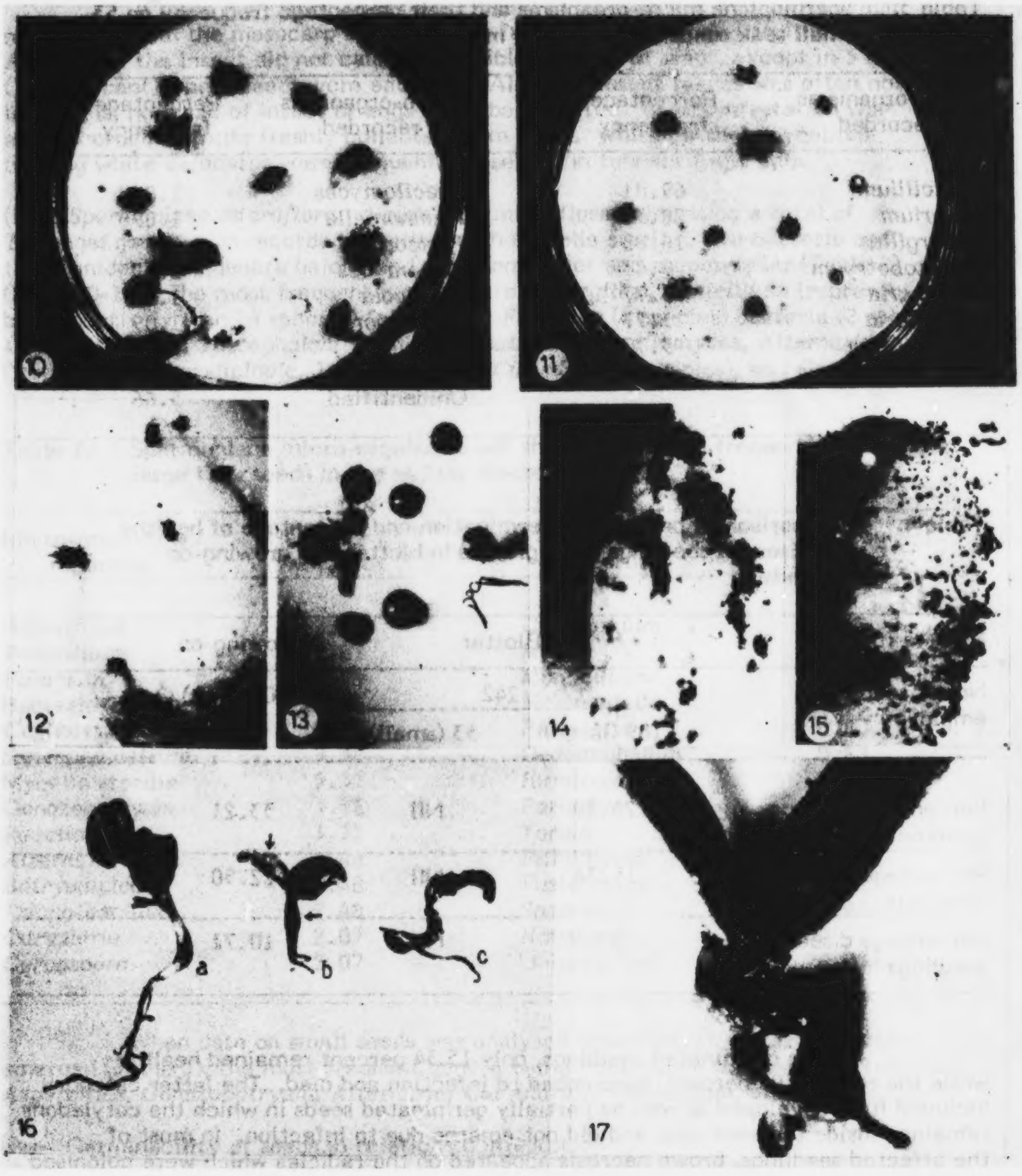
Table 3. Spermoplane micro-organisms and their percentage frequency on 53 small teak seeds in the blotter method.

Micro-organisms recorded	Percentage frequency	Micro-organisms recorded	Percentage frequency
Penicillium	69.81	*Paecilomyces*	1.89
Fusarium	28.30	*Memnoniella*	1.89
Aspergillus	16.98	*Periconia*	1.89
Gonatobotryum	9.43	*Pithomyces*	1.09
Alternaria	3.77	*Humicola*	1.89
Curvularia	3.77	*Cercospora*	1.89
Monocillium	3.77	*Pestalotia*	1.89
Syncephalastrum	1.89	*Chaetomium*	1.89
		Unidentified	5.66

Table 4. Comparison of percentage germination and percentage of healthy and diseased seedlings of *T. grandis* in blotter and growing-on methods.

Method followed	Blotter		Growing-on
No. of seeds employed	242		280 (large)
	189 (large)	53 (small)	
Percentage germination	25.39	Nil	33.21
Percentage healthy seedlings	15.34	Nil	22.50
Percentage diseased seedlings	10.05	Nil	10.71

Of the germinated seedlings, only 15.34 percent remained healthy while the rest (10.05 percent) succumbed to infection and died. The latter category included fully developed as well as partially germinated seeds in which the cotyledons remained inside the seed coat and did not emerge due to infection. In most of the affected seedlings, brown necrosis appeared on the radicles which were colonised by micro-organisms, especially *Fusarium*, *Cephalosporium*, and bacteria (Figs. 16,17). *Aspergillus*, *Penicillium*, *Alternaria* were also recorded frequently on dead seedlings. Seeds which gave rise to healthy seedlings harboured fewer micro-organisms as compared to diseased ones.



Figs. 10-17. ***Tectona grandis.*** Colonisation of seeds with various spermoplane micro-organisms and effect on germination in blotter method (10th day of incubation). 10, large seeds; 11, small seeds; 12, ***Chaetomium***; 13, ***Pestalotia***; 14, ***Pithomyces***; 15, ***Curvularia***; 16, seedlings succumbed to infection: a, necrosis of roots; b, radicle and cotyledons infected with ***Fusarium***; c, seedling killed due to bacterial infection; 17, infected seedling.

b. Growing-on method

Emergence of seedlings started 4-5 days after sowing and continued up to 24 days. However, a large number of them emerged within 10-12 days of sowing. As experienced in the blotter method, the germination of teak seeds in sterile soil was also low (33.21 percent), though slightly higher than on the blotters (Table 4).

The incidence of seedling mortality was comparable in both the methods. Small bits taken from the dead seedlings when plated on PDA (potato dextrose agar medium) yielded pure cultures of 2 species each of *Fusarium*, and *Alternaria* and one each of *Curvularia* and bacterium.

Bombax

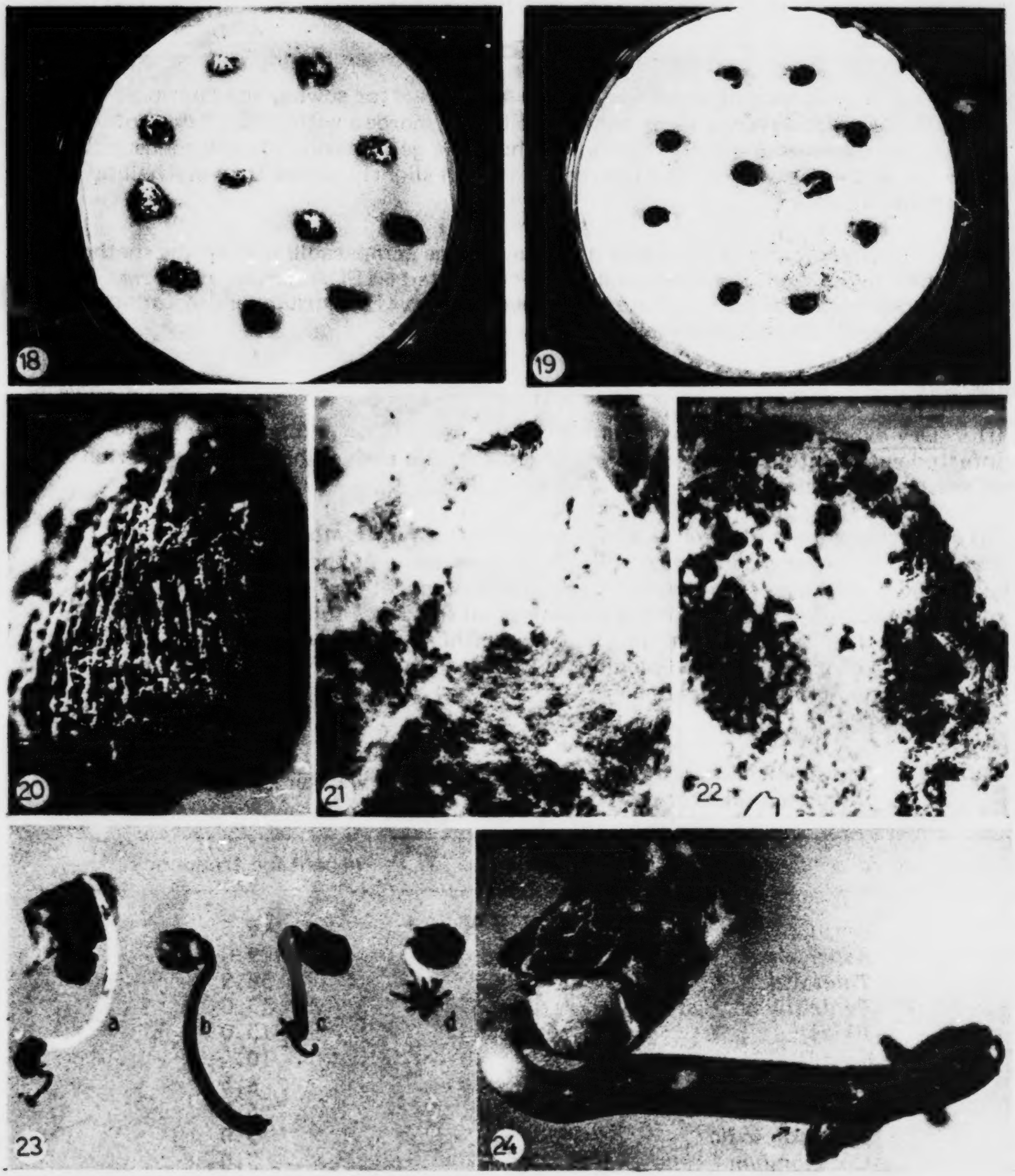
(i) Dry examination - A large number of the seeds (39.5 percent) were badly infested with white and greyish mycelial growth. No shrivelling or discolouration of seeds was noticed.

(ii) *Spermoplane microflora* - Although microflora of *Bombax* seeds was comprised of fewer genera, as compared to teak, some of the micro-organisms exhibited a very high frequency (Table 5). Actinomycetes (3 species) were the most frequent organisms recorded on almost all the seeds followed by *Aspergillus* (5 species), *Thielavia*, *Penicillium* (3 species), *Rhizopus*, bacteria, *Mucor*, *Fusarium* (2 species), *Botryodiplodia* and *Chaetomium*. The two unidentified fungi belonged to hyphomycetes.

Table 5. Spermoplane micro-organisms and their frequency on 200 seeds of *B. ceiba* in blotter method.

Micro-organisms recorded	Percentage frequency
Actinomycetes	163.5
Aspergillus	109.0
Thielavia	58.5
Penicillium	13.0
Rhizopus	13.0
Bacteria	10.5
Mucor	6.5
Fusarium	6.0
Botryodiplodia	3.0
Chaetomium	3.0
Unidentified	5.0

(iii) Germinability of seeds in relation to infection - Considering the fact that *Bombax* seeds were only 7 months old, the germination was very poor (41.0 percent). Most of the ungerminated seeds harboured a dense growth of actinomycetes (Figs. 18-20) and various other fungi (Fig. 22), especially *Aspergillus* and *Thielavia*. The latter produced cottony white mycelial growth which covered the entire seed (Fig. 21). Dark-brown thin-walled ascocarps were produced scattered all over the surface and more profusely at the bottom of seed and on the filter paper. Of the total 43 affected seedlings, except in five where systemic bacterial infection



Figs. 18-24 ***Bombax ceiba.*** Colonisation of seeds with various spermoplane microorganisms and effect on germination in blotter method (8th day of incubation). 18, seeds heavily infected with actinomycetes; 19, germination of only uninfected seeds; 20, small colonies of actinomycetes on seed surface; 21, cottony mycelium and ascocarps of ***Thielavia***; 22, ***Botryodiplodia*** and ***Aspergillus***; 23, seedlings succumbed to infection: a, bacterial infection of cotyledons; b, rotting of radicle and cotyledons inside the testa due to bacterial infection; c, young root infected with actinomycetes; 24, magnified view of 23c. Note colonization of testa with actinomycetes (Act) and ***Thielavia*** (Th) and roots infected with actinomycetes (arrow).

killed the seedlings, 38 were graded as diseased. Generally, infection occurred on radicles. Occasional rotting of cotyledons inside the seed coat was also noticed which resulted in the death of seedlings (Figs. 23, 24).

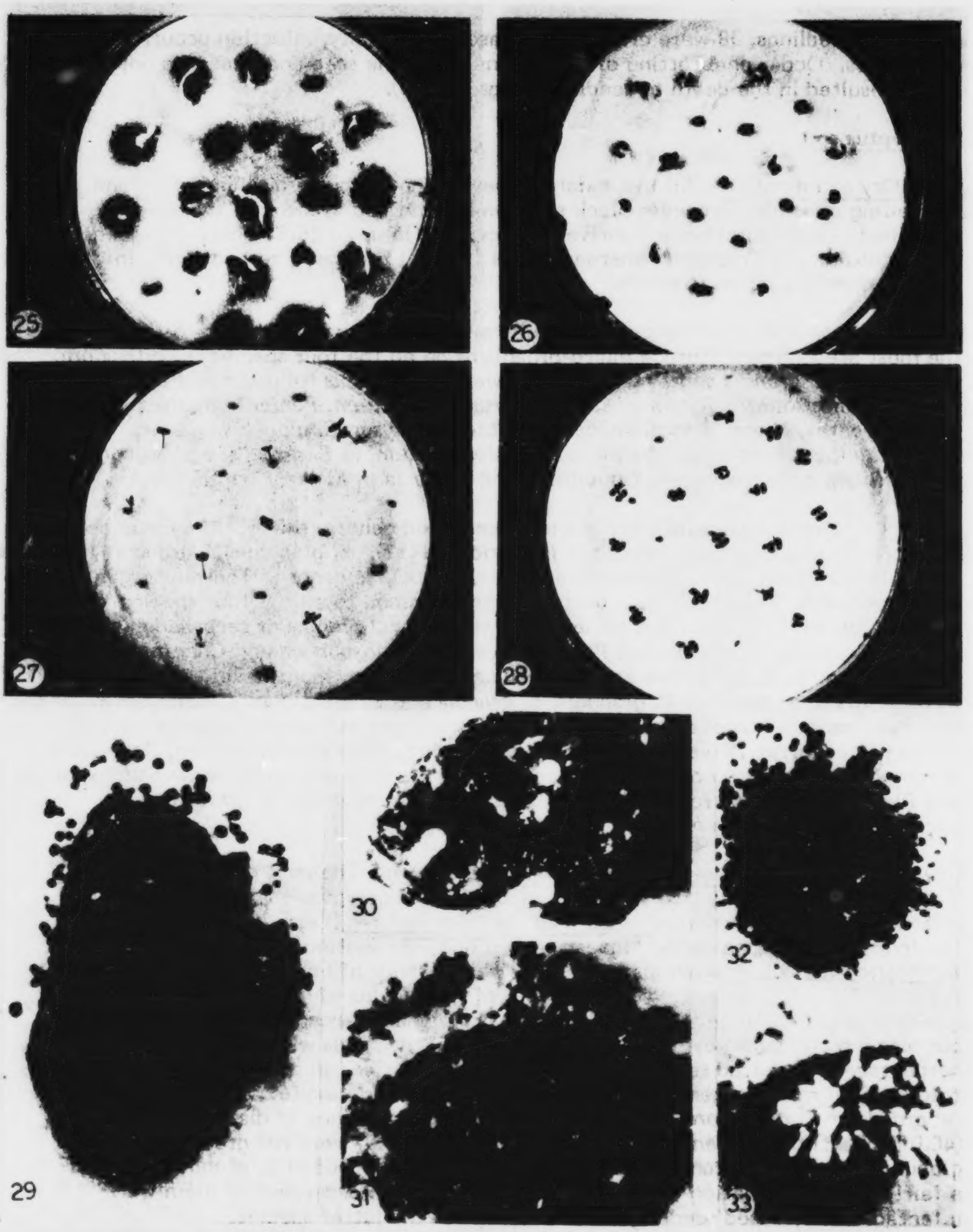
Eucalyptus spp.

(i) Dry examination - All the eucalypt species showed some discolouration and shrivelling of seeds. Brownish black spots were recorded in many of the seeds examined. Discolouration and shrivelling occurred in up to 26-27 percent in seeds of *E. globulus* and *E. grandis* whereas it was 19 and 13 percent, respectively, in *E. citriodora* and *E. tereticornis.*

(ii) *Spermoplane microflora* - The results are summarised in Table 6. As evident the most predominant storage micro-organisms on all the four species *(E. citriodora, E. globulus, E. grandis* and *E. tereticornis)* were *Aspergillus* followed by *Penicillium,* bacteria, and *Colletotrichum* in *E. citriodora; Monocillium, Penicillium,* bacteria, actinomycetes, *Mucor, Paecilomyces* and *Stachybotrys* in *E. globulus; Mucor, Penicillium,* actinomycetes, bacteria, and *Monocillium,* in *E. grandis;* and *Mucor, Monocillium,* actinomycetes, *Penicillium* and bacteria in *E. tereticornis.*

Besides mycelia sterilia and unidentified genera, the maximum number of micro-organisms were recorded in *E. citriodora* (12). *E. globulus, E. grandis* and *E. tereticornis* were colonised respectively by 10,7,6 genera. The four organisms, viz. *Aspergillus, Penicillium* and bacteria were common to all the four species of eucalypt, while others were either common in 2 or 3 species or recorded exclusively from one species. For example, *Paecilomyces, Cephalosporium* and *Chaetomium* were common in *E. citriodora* and *E. globulus,* whereas actinomycetes, and *Mucor* were observed on seeds of *E. globulus, E. grandis* and *E. tereticornis.* On the other hand, a few micro-organisms were associated exclusively to a particular species of eucalypt. Five fungi, viz. *Colletotrichum, Gliocephalotrichum, Phomopsis, Macrophoma* and *Fusicoccum* were recorded only from *E. citriodora* while *Stachybotrys* and *Phoma* were found respectively on seeds of *E. globulus* and *E. grandis.* No exclusive organism was observed in *E. tereticornis.*

(iii) Germinability of seeds in relation to infestation - The data presented in Table 7 indicate clearly that high germinability of eucalypt seeds is well correlated with low infection of seeds with micro-organisms (Figs. 25-33). Among all the four eucalypt species the highest percentage germination was observed in *E. tereticornis* (92.5) which also had the lowest number of micro-organisms (6), recorded from 4.5 percent of seeds. Seeds of *E. globulus* which showed the lowest germination percentage (40.0) had not only the highest percentage of infected seeds but also a high frequency of storage micro-organisms. This was also reflected in the percentage of diseased seedlings recorded in this species. In spite of the high frequency of micro-organisms a reasonably good germination (69.5 percent) was recorded for *E. citriodora.* However, due to a high incidence of diseased seedlings (40.0 percent) the percentage of healthy seedlings (40.0) were not greater than *E. globulus,* which had a considerably low germinability. Seeds of *E. grandis* recorded a fairly good germination (67.0 percent) even though 29.5 percent of them were infected. No diseased seedlings were observed in the latter species.



Figs. 25-33. *Eucalyptus* spp. Colonisation of seeds with micro-organisms and their effect on germination in blotter method (8th day of incubation). 25, *E. citriodora*; 26, *E. globulus*; 27, *E. grandis*; 28, *E. tereticornis*; 29, *Aspergillus* on *E. globulus*; 30, Acervuli of *Colletotrichum* on *E. citriodora*; 31, *Aspergillus* on *E. citriodora*; 32. *Mucor* on *E. grandis*; 33, *Aspergillus* on *E. tereticornis*.

Table 6. **Spermoplane micro-organisms and their percentage frequency on 200 seeds each of *Eucalyptus citriodora*, *E. globulus*, *E. grandis* and *E. tereticornis* in blotter method.**

Micro-organisms recorded	Percentage frequency			
	E. citriodora	*E. globulus*	*E. grandis*	*E. tereticornis*
Aspergillus	55.5 a*	59.0 a	15.0 a	1.5 a
Penicillium	38.0 b	21.5 c	8.5 c	0.5
Monocillium	0.5	23.5 b	1.5	1.0 c
Bacteria	4.5	14.0	4.5	0.5
Paecilomyces	12.0 c	7.5	-	-
Mucor	-	9.0	10.0 b	1.5 b
Actinomycetes	-	10.0	8.0	1.0 c
Mycelia sterilia	3.0	5.5	-	-
Cephalosporium	4.5	3.0	-	-
Stachybotrys	-	4.5	-	-
Colletotrichum	2.5	-	-	-
Chaetomium	1.5	1.5	-	-
Gliocephalotrichum	1.0	-	-	-
Phomopsis	1.0	-	-	-
Phoma	-	-	1.0	-
Macrophoma	0.5	-	-	-
Fusicoccum	0.5	-	-	-
Unidentified	2.5	3.0	0.5	-
Total number of micro-organisms	14	12	8	6

* First three micro-organisms with highest frequency are marked a, b, c.

Table 7. **Germinability (germination percentage) of 200 seeds each of *E. citriodora*, *E. globulus*, *E. grandis* and *E. tereticornis* in blotter method.**

Eucalypt species	*E. citriodora*	*E. globulus*	*E. grandis*	*E. tereticornis*
Percent germination of seeds	69.5	40.0	67.0	92.5
Percent healthy seedlings	29.5	15.5	67.0	92.5
Percent diseased seedlings	40.0	24.5	-	-
Percentage of seeds on which micro-organisms were recorded	69	73.5	29.5	4.5

DISCUSSION

Results presented indicate clearly that seeds of tree species are equally potent in harbouring a rich storage microflora as compared to agricultural and horticultural crops. ***Aspergillus*** was the most predominant fungus in all tree species, except in ***Bombax*** where actinomycetes occurred in very high proportions, followed by ***Penicillium***. Various species of ***Aspergillus*** and ***Penicillium*** which invade and destroy seeds of a wide variety of agricultural, and numerous horticultural crops are already known to constitute the main storage microflora (Neergaard 1977). However, occurrence of actinomycetes on seeds is being reported for the first time. Actinomycetes were also recorded on ***E. globulus***, ***E. grandis*** and ***E. tereticornis***, although with a lower frequency as compared to ***Bombax***. In ***Bombax*** where the seeds were completely covered with this organism, except for ***Penicillium***, ***Aspergillus*** and ***Thielavia***, very few other organisms were recorded. But no indication of antagonism was evident on seeds as reported by Lachance and Parrault (1953) who established that soil-borne actinomycetes exert a more or less pronounced antagonistic effect on ***Fusarium oxysporum*** f. sp. ***lini***. Whether low frequency of ***Fusarium*** on ***Bombax*** as compared to teak where actinomycetes were totally absent is due to actinomycetes needs further investigation.

Unexpectedly, a rich surface microflora was recorded from teak seeds. However, comparatively fewer organisms were observed on ***Bombax*** and all the four eucalypts. A few genera, viz. ***Gonatobotryum***, ***Pestalotia***, ***Humicola***, ***Pithomyces***, ***Torula*** on teak seeds, ***Thielavia*** on ***Bombax*** and ***Gliocephalotrichum*** recorded in spermoplane are possibly the first reports on seeds.

The percentage of seeds on which micro-organisms were recorded was appreciably lower in ***E. tereticornis*** and ***E. grandis*** than in ***E. citriodora*** and ***E. globulus***. Since all the four eucalypt species were stored under similar conditions with BHC treatment, it is not clear whether low frequency of micro-organisms encountered in ***E. tereticornis*** and ***E. grandis*** is due to seed characteristic or some other factor. All the seeds of teak and ***Bombax*** were profusely colonised by micro-organisms.

In addition to common storage organisms, the spermoplane microflora were comprised of numerous potential pathogens, which are also known to be seed-borne in various crops. ***Cercospora***, ***Fusarium*** and bacteria recorded in teak may well be pathogenic since ***Cercospora tectonae*** Stevens (leaf spot), ***Fusarium oxysporum*** Schlect. (mortality in teak seedlings) and ***Pseudomonas solanacaerum*** (E.F. Smith) (vascular wilt) are reported to cause diseases of seedlings or young plants. Other micro-organisms, e.g., ***Alternaria***, ***Curvularia***, ***Botryodiplodia***, ***Cephalosporium***, ***Pestalotia***, ***Chaetomium*** are reported to be pathogenic and cause serious diseases of seedlings in many plant species (Neergaard 1977). Similarly in ***Eucalyptus*** spp., besides ***Fusarium*** and ***Colletotrichum*** which respectively cause wilt and infection of flowers and buds (Sharma and Mohanan, unpublished), ***Cephalosporium***, ***Phoma***, ***Macrophoma*** are potential pathogens. ***Phoma eucalyptica*** has frequently been intercepted on a considerable number of species of eucalypt seeds imported from Australia in the USA (Girard 1973, 1974). Although no occurrence of any pathogenic organisms was recorded in ***Bombax*** seeds, ***Thielavia***, ***Fusarium***, ***Botryodiplodia***, ***Chaetomium*** have potential pathogenic capabilities.

One hundred fruits of teak yielded only 111 large and 29 small seeds, which is in conformity with the earlier observations of Gupta and Kumar (1976), who reported that emptiness, a common feature, varies from 13 to 86 percent in seed samples collected from different parts of India. In the literature no definite reasons are available for emptiness, which consequently leads to poor germinability of teak fruits, probably due to one or more of the following reasons: (i) poor pollination, (ii) common occurrence of abortion of ovules in the family Verbinaceae due to some unknown factor, (iii) seed dormancy and maturity, (iv) abortion of ovules due to infection of field fungi (prior to storage), and (v) infection of storage micro-organisms. Since the first three points are out of the purview of this paper only the last two will be discussed here.

Although it is not of very common occurrence, evidence from work on seeds of cereals suggests that the direct impact of fungi on young developing seed is considerable (Neergaard 1977). Certain parasitic fungi, such as *Fusarium* are particularly pathogenic to flowers and seed primordia, thus reducing seed yield quantitatively. The common occurrence of small and poor-filled shrivelled seeds and failure on the part of these as well as large seeds is possibly due to infection of *Fusarium* which was recorded in high frequency in the blotter as well as the growing-on method. *Fusarium* was also found to colonize profusely the non-germinated seeds in the growing-on method. These observations also confirm a previous report by Mathur *et al.* (1975) who found a strong evidence of correlation between infection of *Fusarium* and loss of germinability of sorghum seeds. Furthermore, sorghum seeds infected with *Fusarium* either did not germinate and if germinated died after emergence.

Under unfavourable conditions of collecting seeds and in storage, storage fungi especially *Aspergillus* and *Penicillium* cause loss of germinability (Christensen 1973). Although teak seeds are enclosed in a stony endocarp, they are frequently observed to be invaded by various types of fungi, including *Aspergillus* and *Penicillium.* This raises a question on the mode of entry of these fungi inside the loculi. Since the endocarp is thick and stony there appears to be less chances of fungus mycelium entering through it. However, it may get entry through two probable points: (i) the pedicel region of the fruit, and (ii) insect tunnels reaching up to the placenta, which is fairly close to the loculi, containing seeds. Seed infection through the peduncle of fruit has been reported by Loughnane *et al.* (1946) who found infection of seeds of flax, still enclosed in capsules, by *Septoria linicola (Mycospheerella linorum).* The pathogen penetrated through the stalk of fruit from the placenta through the funiculus into the seed coat without producing any visible external symptoms on the capsule. The teak seeds may well be getting infected in a similar way, but it needs a detailed investigation to confirm it.

Up to 20 to 30 percent of the teak fruits were observed to be infested with insects, which make extensive tunnels in the mesocarp. Quite often they also penetrate into the endocarp going down up to the placenta. As mentioned earlier these tunnels were colonised by various fungi, especially an unidentified ascomycete. Numerous ascocarps together with loose mycelium were also found on seeds in loculi. The only possibility of it entering is either through the vascular supply of seeds or by direct entry into the loculi.

The poor germinability of teak seeds in blotters was also confirmed by the growing-on method. Slightly higher germination in the latter method possibly resulted either due to a larger number of seeds, or seed dressing with Bavistin, which may have affected the external seed microflora to a considerable extent. Hence the high incidence of seedling mortality may be brought about by only internal seed-borne infection in the absence of external microflora. Isolation of *Fusarium, Alternaria, Curvularia* and bacteria from infected seedlings confirm their pathogenic potential towards the germinating teak seeds. Thus, results obtained in the blotter and growing on methods have shown that the superficial and deep infections (internally seed-borne) are almost equally potent in giving rise to diseased plants. The present study confirms an earlier observation of Dabral (1976) on the involvement of fungi in lowering the germinability of teak seeds. He found that fungal attack on teak seeds was the major problem during germination. The seeds germinated best when the incidence of fungal attack was minimal. When the fungal infection was heavy, even the successfully germinated seedlings did not survive.

Poor germination of seeds and high incidence of diseased seedlings in the blotter method was also observed in ***Bombax*** and ***E. globulus.*** Exceptionally high frequency of actinomycetes and ***Aspergillus*** on ***Bombax*** seeds, and ***Aspergillus*** and ***Penicillium*** on ***E. citriodora*** possibly contributed to poor germination. ***Aspergillus,*** which is known to produce aflatoxin in oily seeds like ***Bombax,*** invade the embryos (Christensen 1973) thus lowering the germinability. Further support on the role of storage micro-organisms in lowering germinability is gained from the work of Sharma and Chauhan (1976) who reported that ***Syncephalastrum, Penicillium, Aspergillus, Paecilomyces*** and actinomycetes ***(Strentomyces*** spp.) are capable of utilizing complex fatty acids by secreting extra cellular lipase, thus causing biodeterioration of oil seeds. Infection of the radicle and young coleoptile by spermoplane micro-organisms was responsible for the high degree of pre- and post-emergence infection in seedlings of ***Bombax, E. citriodora*** and ***E. globulus.*** Post-emergence mortality in ***Bombax*** was mainly caused by bacterial infection.

Discolouration and shrivelling of seeds also affect the germinability to some extent (Christensen 1955). Extensive mycelial growth on the surface was observed only on seeds of ***Bombax.*** In eucalypts only some discolouration and shrivelling but no mycelial growth or fruit bodies were found, except in a few seeds of ***E. citriodora.*** Some of the seed-borne fungi, such as ***Colletotrichum, Fusarium, Cephalosporium, Botryodiplodia,*** recorded in eucalypts are known for causing discolouration of seeds of other crops.

Considering the quantity and varied kind of spermoplane micro-organisms associated with seeds of teak, ***Bombax,*** and four eucalypt species, their possible role in lowering the germinability cannot be denied. The rich seed microflora encountered could be the result of unsuitable storage conditions such as high humidity and temperature which contribute to growth of several saprophytic and pathogenic micro-organisms. These organisms not only cause deterioration resulting in low germinability but what is all the more important is that such seeds are the carriers for introducing potential pathogens in hitherto uninfested areas. This can be overcome only by storing seeds under proper storage conditions and testing the seed health before sowing.

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PROGRESS IN ACORNS TREATMENT IN A LONG TERM STORAGE PROSPECT

by

C. Delatour
Laboratoire de Pathologie forestière
INRA - CNRF (Nancy) Champenoux 54280 Seichamps F.

C. Muller
Station d'Amélioration des Arbres forestiers
INRA - CNRF (Nancy) Champenoux 54280 Seichamps F.

M. Bonnet-Masimbert
Station d'Amélioration des Arbres forestiers
INRA - CRF (Orléans) Ardon 45160 Olivet F.

ABSTRACT

Treatment by heat should kill *Ciboria batschiana* in the infected acorns. Soaking in hot water was more effective than steaming. *C. batschiana* became inviable after 2 to 10 hours in water heated to 38 - 40°C. Acorns tolerated heat well up to 44°C for 8 hours. Some other fungi were also killed (i.e., *Discula umbrinella).* After a 12-month storage at -1°C in dry peat, the treated acorns germinated well (80 - 90%) as compared to the control (40%). These results indicate that thermotherapy might be an effective means to destroy fungi in acorns in storage or destined to international exchanges.

RÉSUMÉ

Un traitement à la chaleur devrait tuer *Ciboria batschiana* chez les glands infectés. Le trempage dans l'eau chaude a été plus efficace que le traitement à la vapeur. On a rendu *C. batschiana* non viable après un trempage de 2 à 10 h dans de l'eau chauffée entre 38 et 40°C. Les glands ont bien toléré la chaleur jusqu'à 44°C pendant 8 h. De plus, d'autres champignons ont été tués (ex.: *Discula umbrinella).* Après 12 mois à -1°C dans de la tourbe sèche, les glands traités ont bien germé (80 à 90%) comparativement aux témoins (40%). D'après ces résultats, la thermothérapie pourrait être un moyen efficace de détruire les champignons chez les glands.

INTRODUCTION

Good oak acorn production occurs every two to ten years, depending on the region. The long term storage of acorns should enable foresters to have viable seeds available every year, and give them the possibility to realize programmed oak reforestation (Bonnet-Masimbert et Muller 1973).

Acorn is a seed relatively hard to preserve because it has to be stored at a high moisture content (45%). Its germinability at low temperatures (below -5°C) is reduced quickly. In addition, acorns may be infected by many microorganisms, especially fungi, which can quickly destroy cotyledons and germs. As previously reported (Morelet 1974), the most important losses observed, are caused by an Ascomycete fungus: ***Ciboria batschiana*** (Zoff) Buchwald responsible for "black rot". This funqus not only can quickly infect acorns (by ascopores, Delatour *et al.* 1977) after their fall in the forest, but also can spread fast, even at low temperatures (-1°C), from one acorn to another by way of its mycelium (Delatour *et al.* 1978). That is how whole crops may be destroyed during their first winter of storage.

The first work performed in Nancy, concerning black rot control, indicated the efficiency of some chemicals, and the best procedure being to soak the acorns over night in fungicide solution of benomyl (0.4 g a.i./1) or methylthiophanate (1 g a.i./1) and then to coat them with TMTD (Bonnet-Masimbert *et al.* 1977). However, the results remained erratic. Later we showed that the classical thermotherapy concept gave better results (Delatour 1978, Delatour et Morelet 1979).

The purpose of this paper is to bring forward new facts concerning the type of thermotherapy and to outline the prospects opened by this technique of acorn preservation.

DIFFERENT TYPES OF THERMOTHERAPY

So far a treatment of dry heat has not been considered due to the relatively high humidity level at which the acorns must be maintained, but, wet heat may be applied either by steaming or soaking in warm water. The latter two treatments have been studied using pure culture of *C. **batschiana*** and artificially infected acorns.

METHODS

a. Trials on pure cultures

Ciboria batschiana was grown in malt-agar culture tubes at room temperature for a period of 15 to 20 days prior to the application of the different treatments. Culture tubes were sealed with a hermetic parafilm for steaming treatment and filled with sterile water for soaking treatment; afterwards these tubes were dipped in water at the desirable temperature.

Survival of *C. **batschiana*** was evaluated after transplantation on new medium or by direct examination for mycelial regrowth in the tubes. Three to ten cultures were assigned to each treatment.

b. Trials on infected acorns

Two lots of 25 acorns of *Q. rubra* were inoculated on their hilum with a mycelial implant taken from the edge of cultures in active growth. After a period of 10 to 15 days, the acorns were treated either by soaking in water at 38°C for 8 hours, or by steaming at 38°C for the same length of time. The acorns were then stored for 6 months in dry peat at 4°C. Afterwards, survival of *C. batschiana* was assessed by the extension of cotyledonary necrosis and mycelium growth on them in moist conditions.

RESULTS AND DISCUSSION

Results presented in Table 1 indicate that *C. batschiana* in pure culture was killed rapidly between 38° and 40°C. The lethal effect of thermotherapy on the fungus seems to occur faster in the case of soaking, but this trend is unclear and does not appear to be similar for all the tested temperatures.

Table 1. Survival of *C. batschiana* in pure cultures after different types of heat treatments

Temperature applied	Treatment type	Treatment duration							
		1/2h.	1h.	2h.	4h.	8h.	16h.	24h.	48h.
34°C	steaming					+	+	+	+
	soaking					+	+	±	±
35°C	steaming			+	+	+	+	+	+
	soaking			+	+	+	-	-	
36°C	steaming			+	+	+	+	+	+
	soaking			+	+	+	±	±	±
38°C	steaming			+	+	+	-	-	-
	soaking			+	±	±	-	-	-
40°C	steaming	+	+	-	-	-			
	soaking	+	+	-	-	-			
42°C	steaming	-	±	-					
	soaking	±	±	-					

Symbols:
+ Survival of all cultures
± Survival of some cultures
- No survival

At 38°C for 8 hours, the pure culture was very close to the survival limits of *C. batschiana*, but apparently there was no difference between soaking and steaming. However, for infected acorns, the steaming is by far less efficient than the soaking (Table 2). This difference may be attributable to an imperfect simulation "in vitro", especially for steaming. One could think that during the steaming, acorns are subject to specific physiological phenomenon resulting in *C. batschiana* in a relative advantage in resistance to heat. The increased evaporation could also allow the fungus to benefit locally of a less lethal temperature.

Table 2. Survival of *Ciboria batschiana* in acorns of *Q. rubra* after heat treatment at 38°C for 8 hours either by steaming or soaking

	Control	Steamed acorns	Soaked acorns
Survival of *C. batschiana*	100 %	80 %	16 %

At the time of soaking the acorns, phenolic compounds, namely tannins are set free into the water and this could reinforce the lethal effect of the properly so called treatment.

Whatever it might be, the soaking appears to be the best method to obtain a total lethal effect on *C. batschiana* between 38°C and 40°C.

THE ACTION OF THERMOTHERAPY ON ACORNS

The thermotherapy evidently did not affect the viability of acorns. We did show previously that acorn viability was affected neither by a 12-hour soaking in water at 38°C nor a 2-hour soaking at 40°C, but it was necessary to perform studies at higher temperature in order to determine the critical threshold and to define a safety margin for treatments.

METHODS

Acorns of *Q. robur* L. were soaked in 40° to 46°C water for 8 hours and their germination percentage determined in the laboratory at 20°C using a vermiculite substrate. Fifty acorns were allotted to each temperature treatment. Moisture content of the treated acorns was also determined.

RESULTS AND DISCUSSION

Our results in Table 3 show that the acorns resist well for 8 hours up to 44°C, beyond that, the germination percentage seems to drop rapidly. This fact proves the hypothesis of a thermotherapy around 38° - 40°C with a relatively important safety margin which are important in the practice.

Table 3. Survival of acorns of *Q. robur* after soaking in water at different temperatures for 8 hours

	Control	Temperature of water						
		40°C	41°C	42°C	43°C	44°C	45°C	46°C
Germination percentage	94	100	94	98	90	92	88	62
Increase of moisture content (%)	0	3.9	3.7	2.5	1.0	1.8	2.3	3.0

ASSESSMENT OF TREATED ACORNS FOR GERMINABILITY AND INFESTATION DURING STORAGE

If the germination percentage of treated acorns measured immediately after the thermotherapy is unaffected, will it be the same after several months of storage? We intend to study this important aspect and the infestation of treated acorns.

METHODS

To examine these aspects, lots of several kg of acorns treated by thermotherapy and by chemotherapy were placed in storage at the National Forest Research Centre in fall 1978. Acorns of *Q. robur* and *Q. petraea* (M.) Liebl. gathered in the neighbourhood of Orléans were free of *C. batschiana*. To obtain an adequate level of infection, artificially inoculated acorns were mixed. Six different treatments were applied as follow:

- control (untreated)
- chemical treatment only (methylthiophanate + TMTD)
- treatment by thermotherapy: 2 steps were used; 10 h at 38°C and 8 h at 39°C, each step subdivided into 2 operations: simple treatment, and treatment with addition of TMTD in the soaking water.

The lots were then stored in dry peat at -1°C.

A sample of 50 to 100 acorns was periodically taken out of each lot for examination. The acorns were decoated, and % infection by *C. batschiana* was recorded. The germination percentage was determined in the laboratory.

RESULTS AND DISCUSSION

The data corresponding to 5 and 12 months of storage is presented in Table 4.

Table 4. **Evaluation of acorn health during storage**

	Duration of storage	Number of observed acorns	Healthy acorns %	C. batschiana %	Germination percentage
Initial conditions			74	2	96
Control	5 months	50	38	36	64
	12 months	100	45	36	40
Methylthio-phanate + TMTD	5 months	100	71	1	87
	12 months	100	65	6	68
38°C/10h.	5 months	50	82	2	84
	12 months	100	81	0	80
38°C/10h. + TMTD	5 months	50	82	0	96
	12 months	100	73	0	84
39°C/8h.	5 months	50	74	2	98
	12 months	100	74	2	84
39°C/8h. + TMTD	5 months	100	73	0	92
	12 months	100	72	0	92

The germination percentage of acorns of the different lots, in the course of time, was compared to that observed in a reference lot of a specially high quality and stored in 1976 (reference lot). This comparison appears in Table 5 in which the germination percentage was measured with reference to the initial germination value (TGo = 100).

Table 5. Germination percentage evaluation comparison between treated acorns and a reference lot (high health condition)

Seed lot and Treatment	initial point	duration of storage			
		6 months	12 months	18 months	30 months
Reference acorn lot	100	99	97	94	84
Studied acorn lot					
Control	100	67	42		
Methylthiophanate + TMTD	100	91	71		
38°C/10h.	100	88	83		
38°C/10h. + TMTD	100	100	88		
39°C/8h.	100	100	88		
39°C/8h. + TMTD	100	96	96		

After one year storage it appears that the health of acorns subjected to thermotherapy, judged on the ratio of healthy acorns, remains close to the ratio at the beginning. The sanitary condition of the lot chemically treated and of the control deteriorated. This assessment has direct repercussion on the germinative capacity of the acorns, which is noticeably higher in the lots treated with thermotherapy with a value near the one of the reference lot. This allows one to expect a satisfactory storage for longer periods exceeding one year.

From the phytosanitary point of view, the germinative capacity of acorns does not depend only on the change of those which are sound at the beginning, but also on the change of those partially infected. The flux of this category may be decisive. In the studied case it represents approximately 25% of the acorns and may be sometimes more important. In this connection it is interesting to note that fungi other than *C. batschiana* are sensitive to thermotherapy, that is how *Discula umbrinella*, (Berk & Br.) Morelet, a fungus present in 10 to 13% of acorns in the control and in the lot treated by chemotherapy, but absent from the lot treated by thermotherapy. The same thing seems to apply to *Phomopsis* sp. *(Phoma endogena ?)* Both are agents of "brown rot".

One question not yet answered is the role played by other fungi, essentially *Penicillium* spp. and mucorales which are taking the place of *C. batschiana* or *D. umbrinella* after thermotherapy. Our observations show that if they have a parasitic activity, it is only very slow, and under any circumstances not conceivable over a period of 12 months.

At last, a surprising fact is the presence of living *C. batschiana* in two cases of thermotherapy. It does not seem to be due to heat resistant strains of *C. batschiana*, as we could observe it from a confirmation test *in vitro*. It could be the result of an unforeseen incident in handling, but considering this fact, it might be wise to slightly increase the treatment's dose (39°C/10 h for example).

GENERAL CONCLUSION

Thermotherapy is more effective than chemotherapy. Very often problems of penetration are encountered with fungicides; the active substance hardly reaches through the host tissues to kill the fungus. This fact may explain the irregularity of some results obtained with chemotherapy.

The results reported here, clearly show that thermotherapy applied by soaking acorns in water, and not by subjecting them to steam, is an efficient method of curative control of *Ciboria batschiana*, and probably also against other fungi such as *Discula umbrinella*. At 39°C for 8-10 hours, the required effect is obtained on *Q. robur*, *Q. petraea* and *Q. rubra*. In addition, there is the benefit of a safety margin of at least 4° to 5°C for *Q. robur*.

Our results have practical application, and this was the reason for us to conduct such a large-scale trial covering 2 tons of acorns *(Q. robur* and *Q. petraea)* was in fall 1979 in cooperation with the French National Office of Forests and the CTGREF (a technical research institute). The first results were encouraging to the extent that we could record the total ellimination of *C. batschiana* after the treatment (39°C/10h) in spite of a bad sanitary condition at the beginning (healthy acorns 30%, *C. batschiana* infested 50 - 60%).

Furthermore, owing to the possible efficiency of the thermotherapy against other fungi, its utilization to reduce risks of parasites introduction for acorns destined to international exchange may be considered. Within this scope, first a thermotherapy can be applied to destroy the insects and a certain number of internal fungal species and then a dressing with a broad spectrum fungicide may be applied as supplementary security, particularly to prevent the fructification of species escaped from the thermotherapy.

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SUMMARY REPORT

Technical Session 3: Effect of insects and diseases on cone and seed storage.

Chairperson: W.H. Fogal
Co-chairperson: D. Chai

H.O. Schooley: Spruce cone beetle (Anobiidae: *Ernobius* Sp.) reduces the natural storage of seed by black spruce *(Picea mariana)*

SUMMARY

A new species of *Ernobius* was discovered in black spruce cones in Newfoundland. This beetle causes significant damage to cones that persist on trees and to those in storage. The beetle is 3 to 5 mm long and medium to dark brown in colour. Flight activity and oviposition begins in mid-July, peaks at the end of July and continues throughout the remainder of the summer. Eggs are deposited between the scales near the base of mature cones from previous season. Pupation occurs next spring. Damage was found in all of 31 locations sampled. As many as 50% of cones in individual collections were damaged. Average damage in any given year did not exceed 18%. The percentage of cone damage increases with storage. For example 14% of cones had emergence holes after 1 year; this rose to 19% in the second year of storage and to 25% after the third year. These figures are only for beetle emergence holes. Detailed study revealed that total damage to cones was 24% by the end of the first, rising to 34% by the end of the third year. More than 3/4 of the seed bearing parts of cones were destroyed in about 63% of the cones from which beetles had emerged. The amount of seed present in infected cones was half that of freshly-collected cones.

DISCUSSION

So far, this beetle has only been recorded in Newfoundland and is not found in other spruces. Chemical control of this insect is not usually required since black spruce cones are not normally stored. It was suggested that insect damage may be due to the high moisture content of the cones, however, it was noted that the beetle is readily adaptable to low moisture content. Cones from both uplands and peatlands are infested by this beetle. Beetle attack has a marked effect on the availability of seeds for regeneration.

C. Delatour, C. Muller and M. Bonnet-Masimbert: Progress in acorns treatment in a long term storage prospect

SUMMARY

Acorns have a high moisture content and cannot be stored below -3°C. During storage, fungal infections cause serious damage. Black rot caused by *Ciboria batschiana* may destroy whole crops after several months of storage. Chemical control of the fungus is erratic but heat treatment (thermotherapy) provides satisfactory control. Because of the high moisture content of the acorns dry heat cannot be used. Soaking infected acorns in hot water at 38-39°C for 8 hrs provides effective control of the fungus during subsequent storage. This treatment has no effect on the germinative capacity of acorns as long as the temperature does not exceed 44°C. There is a safety margin of 5-6°C.

DISCUSSION

Results of these tests are based on germination in the laboratory but germination in the nursery confirms laboratory results up to now. Fungicides such as Benlate can effectively control the fungus when infection rates are small. With high infection rates thermotherapy is best. Acorns are normally collected on the ground. If acorns are collected from the tree the chances of infection may be smaller. However, some infection does occur on the tree.

TECHNICAL SESSION 4: FACTORS AFFECTING SEED STORAGE

Chairperson: S.K. Kamra
Co-chairperson: D.A. Skeates

STORAGE OF *POPULUS MAXIMOWICZII* SEEDS

S. Asakawa

Forestry and Forest Products
Research Institute
P.O. Box 16, Tsukuba Norin Kenkyu Danchi-nai
IBARAKI, 305 JAPAN

ABSTRACT

Without predrying, *Populus maximowiczii* seeds were stored under controlled conditions of temperature and relative humidity. Relative humidity was an important factor at 3°C and 23°C, to maintain the germinability of the seeds. At −17°C, however, their germinability did not vary with relative humidity. The best among the conditions used was the combination of 3°C and 30% relative humidity, under which the seeds of *P. maximowiczii* can retain their germinability with little loss for one year. Those seeds showed very similar behavior under the germination conditions used, either before or after storage.

RÉSUMÉ

Des graines de *Populus maximowiczii* ont été stockées sans préséchage en conditions artificielles de température et d'humidité relative. À -17°C toutefois, celle-ci a cessé de varier avec l'humidité relative. L'optimum se situe à 3°C et à 30% d'humidité relative, où la faculté germinative ne diminue presque pas pendant un an. Dans les conditions de germination utilisées, avant ou après leur stockage, les graines ont eu un comportement fort semblable.

INTRODUCTION

It has generally been said that *Populus* seeds are microbiotic. According to several articles (Busse, 1935; Johnson, 1946; Marcet, 1957; Moss, 1938; Sato, 1949, 1955) dealing with the longevity of *Populus* seeds, however, those seeds may retain their viability for more than one year under appropriate environmental conditions. Most of those articles are well reviewed by Holmes & Buszewicz (1958). Various findings related to the storage of *Populus* seeds are also summarized from the practical point of view (USDA Forest Service, 1974). One of the points recommended is "predrying before storage", but careful handling is required for predrying.

This article is to report the response of *Populus maximowiczii* seeds subjected to different storage conditions without predrying. Another objective was to evaluate the germination behavior of those seeds under different conditions especially after storage.

MATERIAL AND METHODS

Material – *Populus maximowiczii* fruits were collected from one tree in Sapporo, Hokkaido, on June 18, 1963. Those fruits were received at the laboratory in Tokyo, on June 20. After extracting and cleaning, seeds were kept at a refrigerator of 2°C under sealed condition until July 12 of the same year, when they were stored at different conditions.

Moisture content of seeds was 33.8% on dry weight basis (25.3% on fresh weight basis). Germination percent at the beginning of the experiment was as follows:

Temperature	Dark	Light
25°C	64%	70%
20-30°C	68%	70%

Storage condition – Seeds were placed in a small test tube which was covered with cheese cloth. The test tube was placed in a plastic container, together with a flask containing a given concentration of sulfuric acid. The containers were sealed with plastic tape and placed under nine conditions by combining three temperatures of 23°C, 3°C, and –17°C and three relative humidities of 30%, 60% and 90%.

Relative humidity was controlled by the concentration of sulfuric acid, which was determined by specific gravity. Three concentrations of sulfuric acid were prepared at 23°C and used at three temperatures. Therefore, there should have been some differences in actual relative humidity at respective temperatures. According to Wilson (1921), relative humidities (%) at respective temperatures can be calculated as follows:

Storage temperatures	Concentration of sulfuric acid		
	53.4%	38.5%	18.5%
23°C	29.7	59.6	89.8
3°C	27.2	57.7	89.2
–17°C	24.5	55.5	88.6

In this paper, 30%, 60%, and 90% are used for three temperatures, for the sake of convenience.

Germination test – Three replicates of 100 seeds were tested on 1% agar bed in petri dishes for two days under the following conditions: temperature — 25°C and 20-30°C. (20°C for 16 hours and 30°C for 8 hours) and light condition — dark (covered doubly with black paper envelopes just after sowing) and light (exposed to fluorescent light for 8 hours at 30°C). Germination percent was calculated for total seeds, but not for sound seeds.

RESULTS AND DISCUSSION

The change of germination capacity is shown in Table 1. The results are expressed by the percentage to initial germination percent as described in

Table 1. Change in the germination of *Populus maximowiczii* seeds after various periods of storage

Storage condition		Storage period (days)					
Temperature	Relative Humidity	50	100	150	200	250	300
	Germinated at 25°C under light						
	30 %	37	18	3	0	0	9
23 °C	60 %	4	0	0	0	0	0
	90 %	0	0	0	0	0	0
	30 %	79	84	84	96	89	83
3 °C	60 %	87	61	53	39	26	26
	90 %	69	3	0	0	0	0
	30 %	76	76	90	73	73	61
-17°C	60 %	71	73	77	66	71	71
	90 %	74	79	73	61	70	59
	Germinated at 25°C in darkness						
	30 %	36	31	3	0	0	0
23 °C	60 %	2	0	0	0	0	0
	90 %	0	0	0	0	0	0
	30 %	95	100	100	92	91	100
3 °C	60 %	103	73	58	45	33	30
	90 %	70	0	0	0	0	0
	30 %	78	70	83	73	77	59
-17°C	60 %	69	70	63	78	77	66
	90 %	89	84	84	72	81	63

Note: Results are shown by the percentage to initial germination percent.

Table 1 (cont'd)

Storage condition		Storage period (days)					
Temperature	Relative Humidity	50	100	150	200	250	300
	Germinated at 20-30°C under light						
23 °C	30 %	39	20	4	0	0	9
	60 %	7	0	0	0	0	0
	90 %	0	0	0	0	0	0
3 °C	30 %	97	93	81	76	80	76
	60 %	90	70	60	31	20	26
	90 %	76	0	0	0	0	0
-17°C	30 %	67	64	74	66	69	61
	60 %	63	69	76	50	61	69
	90 %	84	64	74	54	64	46
	Germinated at 20-30°C in darkness						
23 °C	30 %	43	15	4	0	0	0
	60 %	3	0	0	0	0	0
	90 %	0	0	0	0	0	0
3 °C	30 %	101	90	93	85	91	88
	60 %	91	72	56	37	28	22
	90 %	67	0	0	0	0	0
-17°C	30 %	85	69	69	69	65	62
	60 %	71	60	78	60	67	60
	90 %	76	66	74	69	76	46

the previous section. Obviously there is a variability in some lots. One reason for the variability may be the presence of empty seeds, and another reason may be the short period for germination. Irrespective of such a variability, however, a clear tendency can be observed.

Either before or after storage, germination behavior of *Populus maximowiczii* seeds does not vary so much with the germination conditions used, although there seems to be some difference due to conditions. The difference may result from germination speed under different germination conditions.

The effect of relative humidity on germination capacity varies greatly with storage temperature (Table 1). At 23°C, the germinability is completely lost after the storage of 50 days under the relative humidity of both 60 and 90%. Under 30% r.h., on the other hand, nearly two thirds of seeds lose their germinability after the same period of storage. At 3°C, 80% or more of seeds continue to retain their germinability for 300 days under 30% r.h. Under 60% r.h., there is a continuous decrease of germinability, but more than 20 percent of seeds still retain their germinability after 300 days. Under 90% r.h., their germinability is completely lost within 100 days. At −17°C, there seems to be no difference among three levels of relative humidity. At this temperature, the decrease of seed germinability is rather slow and nearly two thirds of seeds retain their germinability even after 300 days of storage under any of those three relative humidities. Under 30% r.h., *Populus* seeds seem to retain their germinability better at 3°C than at −17°C.

Table 2 shows the change in seed moisture content after storage under controlled relative humidity. Decrease in seed moisture content under a given level of relative humidity was less with decreased temperatures, which may be due to the difference in the process to reach equilibrium. The result in Table 2 indicates that the level of moisture content may not be directly related to the retention of seed germinability. In this connection, however, it is necessary to conduct the same kind of experiment for longer period, as pointed out by Sato (1949) that the moisture content of 6% on dry weight basis is critical for the longevity of *Populus* seeds.

Table 2. **Change in the moisture content of *Populus maximowiczii* seeds after the storage under different combinations of temperature and relative humidity**

Storage condition		Storage period (days)	
Temperature	Relative Humidity	200	300
	30 %	7.0 %	4.6 %
23 °C	60 %	10.0 %	-
	90 %	-	-
	30 %	8.9 %	6.4 %
3 °C	60 %	12.0 %	10.5 %
	90 %	-	-
	30 %	14.7 %	10.0 %
−17°C	60 %	21.5 %	17.2 %
	90 %	21.0 %	21.0 %

Note: Moisture content is on dry basis.

It is certain that lower relative humidity is better for retaining the germinability of *Populus* seeds both at 3°C and 23°C. In practice, however, it is necessary to use lower temperature in order to maintain good germinability, as emphasized by Sato (1955).

In the case of ordinary cold storage, low relative humidity is required to satisfactorily retain seed germinability. In the case of sub-freezing storage, however, the life-span does not seem to be greatly correlated to the difference in relative humidity although 90% r.h. is somewhat detrimental. In conclusion, the best condition used is the combination of 30% r.h. and 3°C. Under this condition, *Populus maximowiczii* seeds can be stored without any loss of their germinability, even without predrying before storage.

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GERMINATION AND STORAGE OF *SALIX CAPREA L.* AND *POPULUS TREMULA L.* SEEDS

Milan Simak
The Swedish University of Agricultural Sciences
Dept. of Silviculture, Sec. of Reforestation
S-901 83 UMEÅ, Sweden.

ABSTRACT

Fresh collected seeds of *Salix caprea L.* and *Populus tremula L.* were stored at temperatures of ± 0°C, - 5°C and -20°C. Sublots of the seeds were kept at extremely dry conditions *with* $CaCl_2$ (average moisture contents: 2.4% for *Populus* and 4.4% for *Salix* seeds, calculated on a dry weight basis) and alternatively *without* $CaCl_2$ (a.m.c. 7.5% for *Populus* and 8.0% for *Salix*). Furthermore, the seeds *were* stored with cotton (tightly or lightly packed) and alternatively *without* cotton. The best result after 44 months storage was reached at a temperature of -20°C, without $CaCl_2$-drying. It appears most preferable to store the seeds *without* cotton in tightly closed glass-tubes or in ampoules closed by melting.

Great effort was expended to clarify what is a "normally germinated seed" in sallow and aspen. The criteria concerning this subject in the literature vary widely, which causes difficulties when comparing the results of different storage experiments. On the basis of comprehensive analyses on germinated sallow and aspen seedlings raised from fresh and stored seeds, a definition for a "normally germinated seed" was proposed.

RÉSUMÉ

Des graines de *Salix caprea L.* et de *Populus tremula L.* venant d'être recueillies ont été entreposées à des températures de ± 0°, -5° et -20°C. Certaines ont été conservées dans des conditions extrêmement séches en présence de $CaCl_2$ (teneur moyenne en humidité (masse séche) de 2,4% pour les graines de *Populus* et de 4,4% pour celles de *Salix*) et d'autres en l'absence de $CaCl_2$ (humidité moyenne de 7,5% pour *Populus* et de 8,0% pour *Salix*). Des graines ont, de plus, été entreposées avec du coton fortement ou faiblement tassé) et d'autres sans coton. Apres 44 mois d'entreposage, les meilleurs résultats ont été obtenus à une température de -20°C, sans dessiccation en présence de $CaCl_2$. Il semble préférable de conserver les graines sans coton dans des tubes en verre bien fermés ou dans des ampoules scellées par fusion.

De grands efforts ont été consacrés à la détermination de ce qui constitue une "graine germée normalement" pour le saule Marsault et le tremble d'Europe. Les critères à cet égard varient grandement selon les publications, ce qui cause des difficultés lors de la comparaison des résultats des différentes expériences d'entreposage. A partir d'analyses approfondies des semis de saule et de tremble obtenus au moyen de graines fraîches et entreposées, une définition d'une "graine germée normalement" a été proposée.

INTRODUCTION

In recent years both *Salix* and *Populus* have become interesting species for short rotation forestry as potential producers of "alternative energy" (Sirén 1979).

For this purpose a search is on for fast growing *Salix* and *Populus* species and individuals. A genetic improvement program is under way in which crossing experiments with the selected materials play an important role. In this connection arise many problems related to the seeds obtained from these experiments. Information about the seed biology can be found mostly in botanical literature (Nilsson 1928, Kobendza 1952, Nikisin 1958, Maljutina 1974, 1975 etc.). A good literature survey concerning processing, storing, sowing etc. of the seed was undertaken by Suszka (1973), Brinkman (1974) and Schreiner (1974).

A practical problem of particular importance is how to retain the viability of the seeds that have to be stored for several years. It is well-known that the seeds of *Populus* and *Salix* species lose all viability very quickly under natural conditions, within a few weeks. Also in artificial storage the viability of these seeds is relatively instable (cf. Table 1).

As Schreiner (1974) emphasized, there are difficulties in comparing the results of different seed storage experiments, because many authors have failed to define their criteria or have used their own criteria for what is a "normally germinated seed" in sallow or aspen.

The object of this study was firstly to investigate and describe the morphologically normal and abnormal seedlings of *Salix caprea L.* (sallow) and *Populus tremula L.* (aspen) in order to establish proper criteria for a "normally germinated seed" and secondly, to apply these criteria for evaluation of the result of a storage experiment with these two species.

MATERIAL AND METHODS

Collection and storage conditions. Twigs with catkins were collected near Stockholm (Lat. 59°20'N) at the time when the natural seed dispersal began (T2.06.76). The twigs were dried overnight in a room for seed extraction at 35°-40°C. Next morning the extracted seeds were stored in the following way:

H: Seeds with cotton were tightly packed in glass-tubes ("Hard-pack")
L: Seeds with cotton were lightly packed in glass-tubes ("Light-pack")
O: Seeds separated from the natural cotton were stored in glass-tubes or ampoules.

The seeds were separated from the cotton using the air stream method (Einspahr and Schlafke 1957).

The three alternatively packed seed series were stored in jars or ampoules

1. with $CaCl_2$
2. without $CaCl_2$

The jars and ampoules were stored at three different temperatures as shown in Figure 1:

POPULUS TREMULA L.

Author(s)	Storage conditions		Length of storage (days)	Germination percentage
	Temperature (degrees C)	Others		
REIM 1930		Fresh collected seeds	0	(95)
	T_R		79	73
	T_C	Seeds with cotton, not dried		42
	"	Seeds with cotton, dried		35
	"	Seeds without cotton, dried		83
BUSSE 1935		Fresh collected seeds	0	100
	T_R	In vacuum, 1 mm Hg	278	76
	"	In vacuum, 1 mm Hg	443	5
	"	In vacuum, 1 mm Hg	670	2
	T_R	In vacuum, 40 mm Hg	278	30
	"	In vacuum, 40 mm Hg	443	11
	"	In vacuum, 40 mm Hg	670	0
	T_R	Without vacuum	278-670	0
	T_C*	In vacuum, 1 mm Hg	278	93
	"	In vacuum, 1 mm Hg	443	85
	"	In vacuum, 1 mm Hg	670	84
	T_C	In vacuum, 40 mm Hg	278	88
	"	In vacuum, 40 mm Hg	443	89
	"	In vacuum, 40 mm Hg	670	0
	T_C	Without vacuum	278-670	0
WETTSTEIN, W.V. 1936		Fresh collected seeds	0	(99)
	first T_R		12	70
	then 5	In cellar	30	16
	then 5	In cellar	60	1
MARCET 1957		Fresh collected seeds	0	99
	T_R*	In vacuum, with cotton on the seeds	58 204	30 0
	T_R	In vacuum, without cotton on the seeds	58 204	66 0
	2-3	In vacuum, with cotton on the seeds	1106	77
	2-3	In vacuum, without cotton on the seeds	1106	71
BUC 1969		Fresh collected seeds	0	100
	-2 to -5	Solutions: over $MgCl_2$ and CH_3COOK m.c. of seeds 4-6%	365	98
	6 to 8	over $Ca(NO_3)_2$, m.c. of seeds 5.8%	365	26
		" $MgCl_2$, seeds 4.2%	365	88
		" CH_3COOK seeds 3.6%	365	96
		" $CaCl_2$ seeds 3.6%	365	98
KREBES and CASNOCHA (cit. Ancak 1972)		Fresh collected seeds	0	(?)
	20	Freeze drying	180	79-85

SALIX CAPREA L.

Author(s)	Temperature (degrees C)	Others	Length of storage (days)	Germination percentage
BUC 1960		Fresh collected seeds	0	100
	-2 to -5	over $MgCl_2$ + CH_3COOK	365	98
KREBES and CASNOCHA (cit. Ancak 1972)		Fresh collected seeds	0	(?)
	20	Freeze drying	180	79-85
KOVAC and LAZAREVIC 1976		Fresh collected seeds (April 23-24)	0	(97)
	T_R	Storage	60	20
	±0	Storage	130	100
		Fresh collected seeds (May 1)	0	(100)
	T_R	Storage	60	1
	±0	Storage	120	100

*T_R = room temperature *T_C = cellar temperature

Table 1. Some data from earlier storage experiments with *Salix caprea L.* and *Populus tremula L.* seeds.

1. ± 0°C 2. -7.5* to -5.0°C** 3. -15* to -20°C**

for simplicity indicated as ±0°, -5° and -20°C respectively. Thus the experiment was comprised of 18 different storage conditions for each of the two species (3 "packing" x 2 "drying" x 3 "temperatures"). At each temperature were stored 4 glass-jars *with* $CaCl_2$, 4 glass-jars *without* $CaCl_2$ and the ampoules in order to obtain a sealed seed material for each of the four planned germination tests. The number of ampoules had to be reduced due to the shortage of seeds.

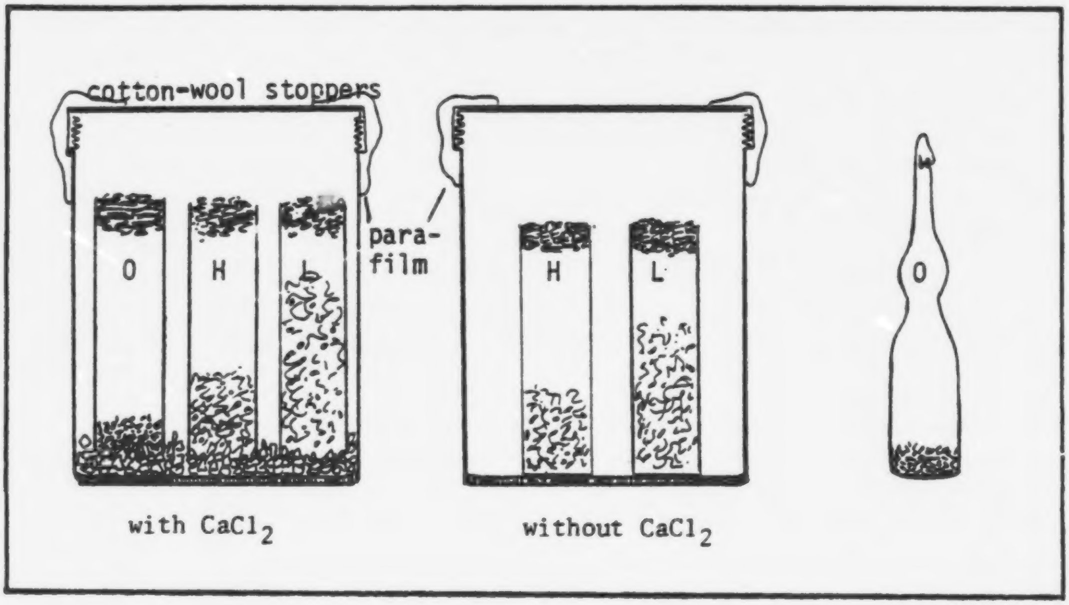

Figure 1. Each glass-tube with the seeds was closed by a piece of cotton-wool and placed in a glass-jar *with* and alternatively *without* $CaCl_2$. In one glass-jar were stored the glass-tubes of both the species; 6 and 4 glass-tubes/jar respectively. (In Figure 1 only glass-tubes of one species per glass-jar have been drawn.). The jars were tightly closed by a cover and sealed by a parafilm. The seeds of the 0-series *without* $CaCl_2$ were placed in glass ampoules then predried for one day over $CaCl_2$ and closed by melting.

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* temperatures at Stockholm ** temperatures at Umea

A disturbance occurred on the 26th month of the storage, when the seed material was being transported from Stockholm to Umea in connection with the moving of the Forestry College. On this occasion all the experimental material was stored for two months at -5°C before the glass-jars were placed again in the respective temperature conditions as given above.

Moisture content of the seeds, (m.c.). At each test occasion a small amount of seeds from the 0-glass-tubes (*with* $CaCl_2$) and from the ampoules (*without* $CaCl_2$) were used for determining the moisture content in the seeds stored *without* cotton. These seeds, after being weighed, were dried at 105°C for 16 hours. The amount of water lost through evaporation was expressed in percent on the basis of dry substance weight of the seeds.

Average moisture contents of the seeds (for all storage temperatures and years):

	with $CaCl_2$	*without* $CaCl_2$
Salix caprea L.	4.4%	8.0%
Populus tremula L.	2.4%	7.5%

In one of the ampoules the moisture content accidently increased during the storage to 19.5% (44 months storage at -20°C *without* $CaCl_2$, series 0). This value is not included in the average above. The moisture content of the seeds stored *with* cotton was not determined. It is probably slightly higher than that of the seeds *without* cotton.

Testing of the seed germinability. The germination percentage of the seeds, directly after collection was for *Salix* 99% and for *Populus* 97.5%. The germinability of the stored seeds was tested on Jacobsen's apparatus at a constant temperature of 20°C and constant light intensity (1000 lux) after 13, 24 and 44 months storage (Figure 2). However, only the results after 24 and 44 months storage are presented, as the first years test-material was used for different studies of the germination process.

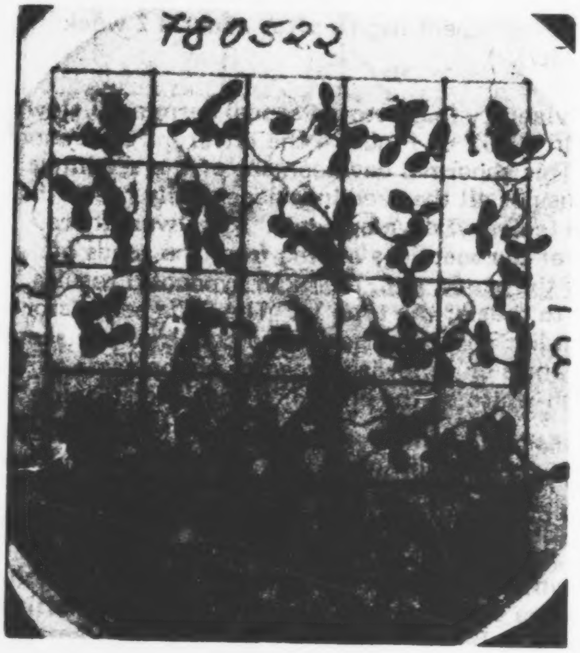

Figure 2. Seedlings of *Salix caprea L.* on the blotter as seen from above. 4 x 75 seeds from each storage-series were used for a test. Each replicate of 75 seeds was placed on a blotter on which a figure of 25 squares (5 x 5) was drawn. Placing 3 seeds per square using a fine brush, each seed or seedling could be kept under control during the whole test.

Evaluation. Various observations of the germinated seeds were performed after 1, 2, 3 and 14 days of the germination test. Classification of the seedlings was carried out on the 7th and 21st day of the germination test according to the system described in Figure 4. The root development was studied in transparent light on a glass-table. In this way the root of each seedling was clearly recognizable and could be properly classified. No harm was caused to the seedlings by the occasional transfers of the blotters between the germinator and the glass-table.

RESULTS

Course of germination. The different germination stages of *Salix caprea L.* and *Populus tremula L.* are described in Figure 3. The course of germination of aspen is similar to that of sallow, but the former is about one day delayed (in the present material).

Sallow and aspen seeds are very small; length of a seed is about 1 mm, and 1000 seed weight is about 0.06-0.2 g (cf. Reim 1930). A seed contains in reality only a large embryo, surrounded by a transparent thin coat. Endosperm is not present except for a few cells (cf. Håkansson 1954). The main parts of a well developed seed are the thin coat, cotyledons, hypocotyl-ring and radicle (stage 1a, Figure 3:1 and 3:8). A viable seed becomes green a few hours after commencement of germination. Within the first day the hypocotyl increases in length and diameter, shows a positive geotropic bending (stage 2a, Figure 3:2), and on the hypocotyl ring arise the radial growing hairs which attach the seedling to the substrate (stage 3a, Figure 3:3). Thereafter, the elongating hypocotyl raises up into a vertical position (stage 4a, Figure 3:4) and the main root begins to grow out. The seed coat bursts when the hypocotyl increases in length. On the second day the seed coat is stript off by the emerging cotyledons. Stage 5a is reached (Figure 3:5). The main root is fast developing and is well visible on the third day (stage 6a, Figure 3:6). The epicotyl development begins after about a 2 week germination period (stage 7a, Figure 3:7).

Seeds possessing a lower viability (e.g., stored seeds) germinate slowly and often abnormally (cf. v. Wettstein 1936). In Figure 4 the different seed abnormalities are classified in a system. This abnormal development of the seedlings can be arrested at any one type. Thus not all the seedlings necessarily reach the final type "f" of the respective stage (epicotyl development). Moreover, there is often a great variation in the size of the seedlings arising from the seeds of low viability. Mechanical damage to the seeds, e.g., a broken hypocotyl results in an abnormal seedling very similar to that of the type 1c. The *Salix* seeds stored for 44 months sometimes showed peculiar abnormalities in the colour of the seedlings. They could be completely yellowish-white or yellowish-green or at the same time dark green in the centre and yellowish-white on the edge of a cotyledon (Figure 3:12).

Storage experiment. The results of the storage experiments for *Salix caprea L.* and *Populus tremula L.* are presented in Figure 5, Table 2, and Figure 6, Table 3 respectively. The following conclusions can be drawn:

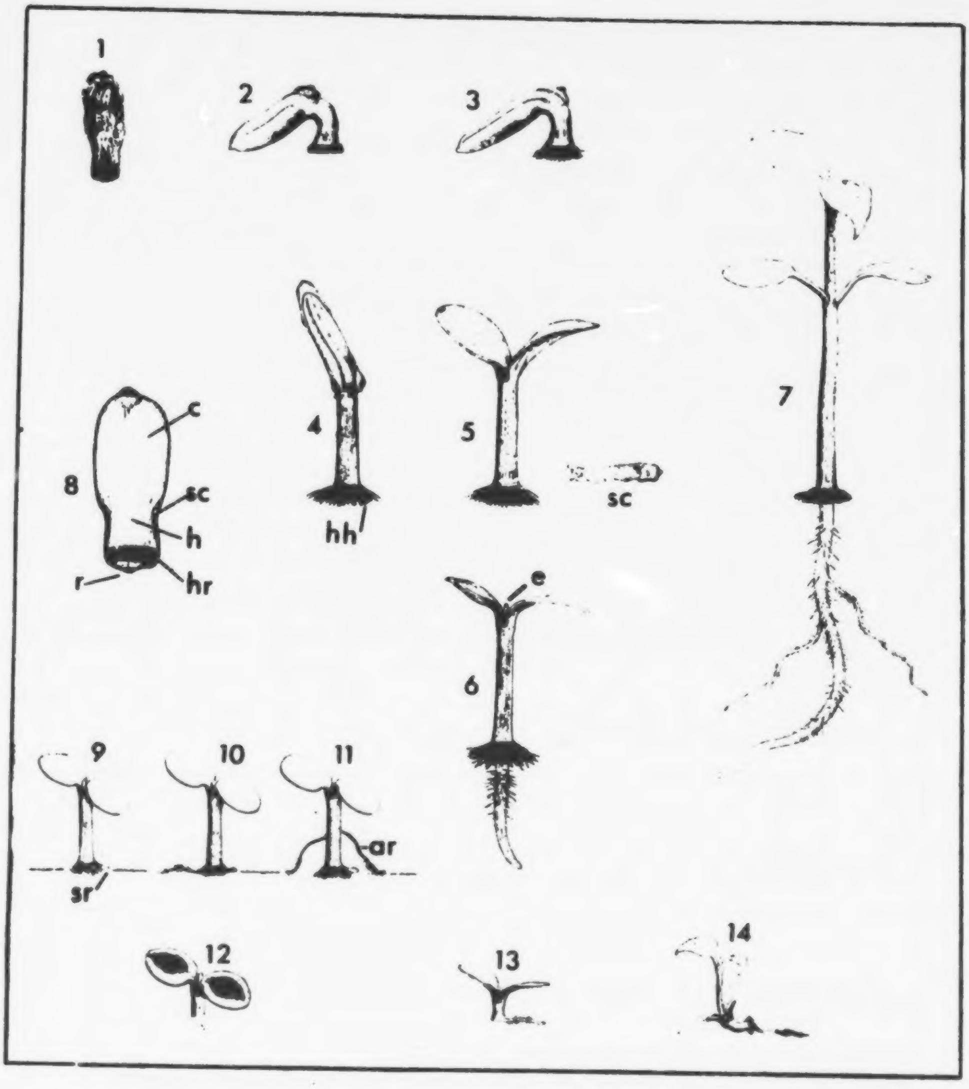

FIGURE 3. *Salix caprea L.* 1-7: Normal development of a seedling. Stages 1a - 7a are indicated according to Figure 4. Description is given in the text. 8: Parts of a seed: c = cotyledons, h = hypocotyl, hr = hypocotyl ring, r = radicle, sc = seed coat. 9-14: Abnormal seedlings. 9: Seedling with stumpy root (st. 5c), 10: Seedling with stumpy root from which an adventive root is growing out (st. 5d), 11: Seedling with stumpy root and two adventive roots growing out from the hypocotyl (st. 5c), 12: Seedling with discoloration; centrum of a cotyledon is green, edge of the same is yellowish-white, 13: hypocotyl is not elongated and shows no radial hairs. Main root (adventive?) is normally growing. The seedling is in vertical position of accidentality (st. 1e), 14: bent and leaning hypocotyl with hairs and a normally developing main (adventive?) root and epicotly (st. 3f).
ar = adventive root, e = epicotyl, hh = hypocotyl hairs, sr = stumpy root.

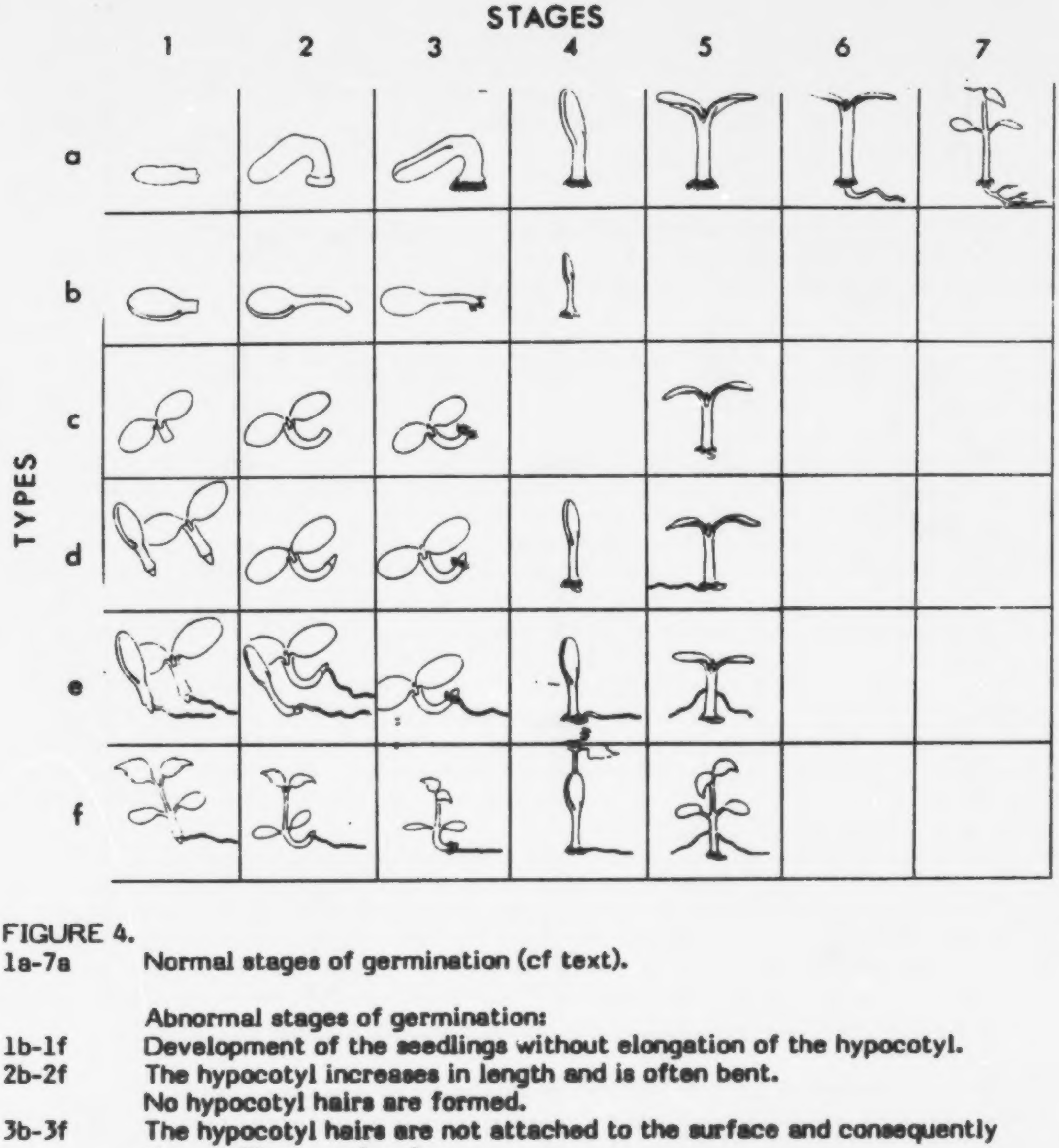

FIGURE 4.

1a-7a	Normal stages of germination (cf text).
	Abnormal stages of germination:
1b-1f	Development of the seedlings without elongation of the hypocotyl.
2b-2f	The hypocotyl increases in length and is often bent. No hypocotyl hairs are formed.
3b-3f	The hypocotyl hairs are not attached to the surface and consequently the seedlings are leaning
4b-4f	The cotyledons are prevented from emerging by the tightly fitting coat.
5c-5f	The main root stopped its growth after reaching a length of 1-2 mm. "Stump roots". Adventive roots, especially of aspen, can grow out from the hypocotyl or the stump-root.

The stump, adventive, and main roots are drawn in the figure in horizontal direction as they are growing on a blotter.

SALIX CAPREA L.

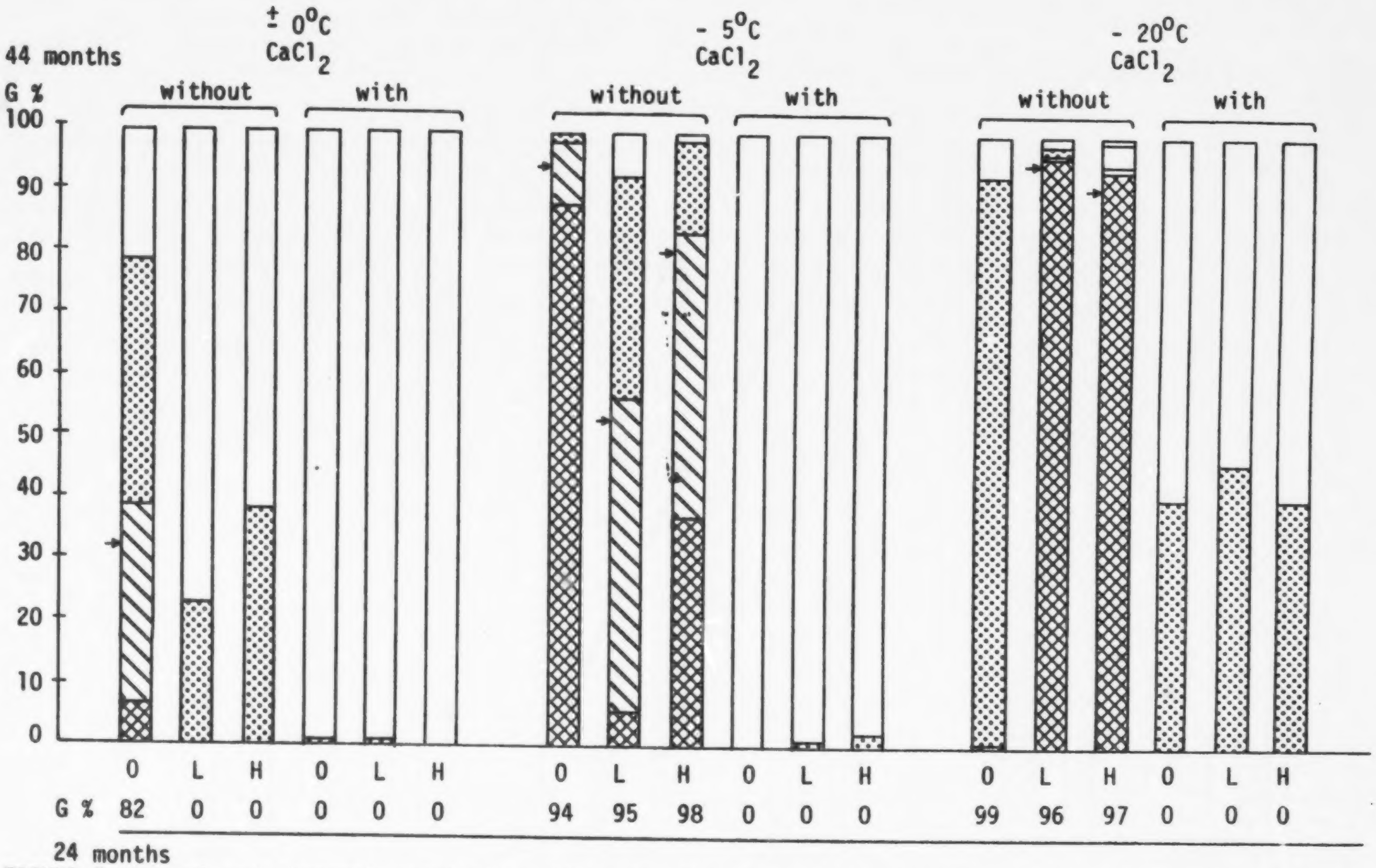

FIGURE 5. Normal and abnormal germination of seeds after 24 and 44 months storage at different conditions. G % = germination percent after 21 days, → indicates germination at the 7th day. Normally germinated seeds ▩ stage 7a, ▨ stage 6a. Abnormally and not fully germinated seeds ▒ Concerning details on abnormally germinated seeds see Table 2 below and Figure 4.
Non germinated seeds ☐

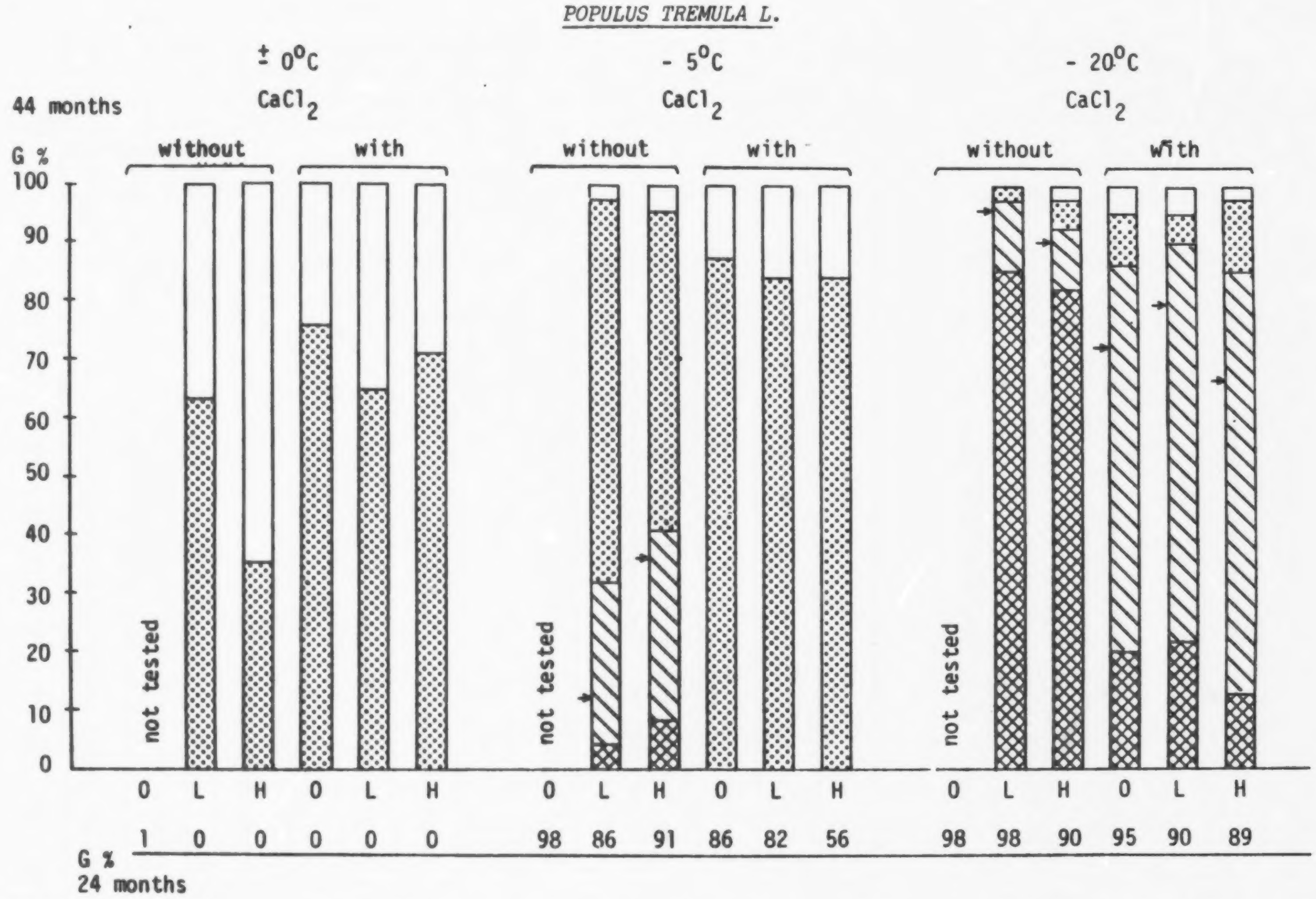

FIGURE 6. Normal and abnormal germination of seeds after 24 and 44 months storage at different conditions. G % = germination percent after 21 days, → indicates germination at the 7th day. Normally germinated seeds ▩ stage 7a, ▧ stage 6a. Abnormally and not fully germinated seeds ▒ Concerning details on abnormally germinated seeds see Table 3 below and Figure 4. Non germinated seeds ☐

SALIX CAPREA L.

Storage temperature and other conditions / type of germination	± 0°C						-5°C						-20°C					
	without CaCl$_2$			with CaCl$_2$			without CaCl$_2$			with CaCl$_2$			without CaCl$_2$			with CaCl$_2$		
	O	L	H	O	L	H	O	L	H	O	L	H	O	L	H	O	L	H
1a	21.6	77.3	62.0	99.7	99.7	100.0	-	7.0	1.1	100.0	99.0	97.7	6.6	1.4	0.4	59.3	53.4	59.4
1b-f	18.7	16.0	19.7	0.3	0.3	-	0.3	18.7	8.3	-	1.0	2.3	0.7	0.3	1.7	24.0	27.3	20.3
2a-f	3.0	2.0	3.0	-	-	-	1.0	4.0	4.0	-	-	-	2.0	-	0.3	16.7	19.3	20.3
3a-f	0.7	4.0	4.3	-	-	-	-	2.3	0.3	-	-	-	2.3	-	0.7	-	-	-
4a-f	6.7	-	1.0	-	-	-	-	2.0	1.3	-	-	-	-	0.7	0.3	-	-	-
5a-f	10.3	0.7	10.0	-	-	-	-	8.0	0.3	-	-	-	87.0	-	-	-	-	-
damage	0.7	-	-	-	-	-	0.3	-	0.7	-	-	-	0.7	-	1.3	-	-	-
6a	32.0	-	-	-	-	-	9.7	52.3	46.7	-	-	-	0.7	0.3	0.3	-	-	-
7a	6.3	-	-	-	-	-	88.7	5.7	37.3	-	-	-	-	97.3	95.0	-	-	-
Germinated seeds 6a & 7a	38.3 ±5.06	-	-	-	-	-	98.4 ±1.03	58.0 ±4.82	84.0 ±1.29	-	-	-	0.7	97.7 ±0.87	95.3 ±0.29	-	-	-
Seedling colour 5a&6a&7a	Y+G+ +G/Y	Y	Y	-	-	-	G+Y+ +G/Y	G+Y	G+Y	-	-	-	G+Y	G	G	-	-	-
Hypocotyl-length ≈mm 6a&7a	5v	-	-	-	-	-	7v	6v	6v	-	-	-	4v	10	9	-	-	-
Root length ≈mm 6a&7a	6	-	-	-	-	-	>10	10	>10	-	-	-	5	>10	>10	-	-	-

TABLE 2. Normal and abnormal germination of seeds after 44 months storage at different conditions.
Percentages after 21 days germination.
O = without cotton; L = with cotton, lightly packed; H = with cotton, hard-packed.
Y = yellow; G = green; Y+G = most of the plants yellow, the rest green; G+Y = most of the plants green, the rest yellow;
G/Y = the central part of a cotyledon is green, the marginal part yellow; v = great variation in the length.

POPULUS TREMULA L.

Storage temperature and other conditions / type of germination	$\pm 0^{o}C$					$- 5^{o}C$					$-20^{o}C$				
	without $CaCl_2$		with			without $CaCl_2$		with			without $CaCl_2$		with		
	L	H	0	L	H	L	H	0	L	H	L	H	0	L	H
1a	36.7	64.9	24.0	35.0	28.9	2.3	4.6	12.6	15.7	16.0	0.4	2.7	5.0	5.1	2.3
1b-f	29.3	21.7	30.0	22.0	18.0	6.0	7.0	16.7	14.0	12.0	0.7	3.7	5.0	2.7	7.0
2a-f	0.7	-	-	-	-	-	-	-	-	-	-	0.3	-	0.3	0.3
3a-f	0.3	1.0	1.0	1.0	1.7	1.0	-	0.7	0.3	1.3	0.7	0.3	-	0.3	0.7
4a-f	5.0	1.7	3.7	2.3	6.0	0.7	1.7	3.0	4.3	8.0	0.3	0.7	1.3	1.3	2.7
5a-f	28.0	10.7	41.0	39.7	44.7	58.0	45.7	67.0	65.7	62.7	0.3	-	2.0	0.3	1.7
damage	-	-	0.3	-	0.7	-	-	-	-	-	-	-	-	-	-
6a	-	-	-	-	-	28.0	32.7	-	-	-	12.3	10.3	66.7	68.3	72.3
7a	-	-	-	-	-	4.0	8.3	-	-	-	85.3	82.0	20.0	21.7	13.0
germinated seeds %, 6a&7a	-	-	-	-	-	32.0 $\pm$2.83	41.0 $\pm$0.85	-	-	-	97.7 $\pm$0.87	92.3 $\pm$1.19	86.7 $\pm$1.89	90.0 $\pm$2.35	85.3 $\pm$1.31
hypocotyl-length ≈mm 6a & 7a	-	-	-	-	-	5v	5v	-	-	-	10	9	8v	8v	8v
root length ≈mm, 6a&7a	-	-	-	-	-	3	5	-	-	-	>10	>10	>10	>10	>10

TABLE 3. Normal and abnormal germination of seeds after 44 months storage at different conditions. Percentages after 21 days germination.

0 = without cotton; L = with cotton, lightly packed; H = with cotton, hard-packed.

v = great variation in the length.

Salix caprea L.

1 There is a clear trend that the lower the storage temperature, the better the seed viability is preserved. The optimum storage temperature is -20°C.

2 Seeds stored in the presence of $CaCl_2$ lost their viability after two years' storage in all three temperature-series*.

3 Seeds stored 44 months at ± 0°C and - 5°C and with the absence of $CaCl_2$ preserved their viability better if stored *without* cotton (0-series in ampoules) than those stored *with* cotton (L- and H-series in the glass-tubes). The ampoule stored for 44 months at a temperature - 20°C was accidently damaged, probably during the transport from Stockholm to Umeå. Herewith increased the m.c. to 19.5% and consequently the seed viability was destroyed.
Nota bene this 0-series after two years storage showed the best germinability (99%). Probably, if the ampoule had not been broken, this series would give the best result after 44 months storage.

4 The percentages of germinated seeds after 7 days test are nearly identical with those of 21 days. The higher the final germination percentage of a sample, the smaller is the difference between the germination percentages of 7th and 21st day.

5 Some seed samples possess a high frequency of abnormally germinated seeds. The abnormalities occur in the morphology and/or in discoloration of the seedlings.

Populus tremula L.
Aspen and sallow seeds have about the same requirements for optimum storage concerning temperature and humidity. Also the germination pattern after 7 and 21 days as well as the occurrence of morphological abnormalities are similar in both the species. However, there are some small differences between the two species in the behaviour of the stored seeds. In contrast to sallow, the aspen seeds stored for 44 months at - 20°C *with* and *without* $CaCl_2$ showed almost identical germinability after 21 days (cf. Table 3). On the other hand, there is a clear difference in the aspen-seedling quality between the two series: the frequency of seedlings belonging to stage 7a is higher and the seedling development better in the series *without* $CaCl_2$. Stored aspen seeds usually germinate better than sallow seeds but the seedlings are not vigorous due to morphological abnormalities. Chlorosis of the plants as observed in sallow, did not occur in aspen seedlings. Finally, the epicotyl develops slower on aspen seedlings than on sallow.

Unfortunately, no aspen seeds were available in the series 0 - *without* $CaCl_2$ after 44 months storage. Some idea about what the germinability of these seeds would be like, is given by the results of two years' storage; it was the best of all (Figure 6).

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* As a matter of fact the seeds in these series were already dead after one year's storage.

DISCUSSION

Course of germination

The course of seedling development of *Salix caprea L.* and *Populus tremula L.* presented in this study agrees in general with the descriptions given by earlier workers (Lagerberg 1922, Kobendza 1952, Nikišin 1958, Maljutina 1975 etc.). A special effort was made to define the concept: what is a "normally germinated seed"? Usually a sallow or an aspen seed is considered "germinated" when "the hypocotyl has grown out of the seed coat just far enough to raise one end of the seed from the substratum" (Faust 1936, Sato 1949). Popcov and Buč (1952) use the same definition with the addition that the hypocotyl hairs have to be attached to the surface. According to Moss (1938) the seed is germinated "when the sturdy hypocotyl bears the cotyledons well aloft within the first two days" of the germination test. These and other similar definitions may be correct as far as freshly collected seeds of full viability are concerned. However, the definitions can be misleading when working with stored, less viable seeds. For instance, according to the definitions above, the sallow and aspen seedlings belonging to the germination types 5a-f (cf. Figure 4 and Tables 2 and 3) have to be classified as germinated. In reality they are not vigorous, lacking the main roots or possessing only 1-2 mm long stumpy roots. They remained in this stage in the germinator for more than two months (after which time the experiment was discarded). Some of them could eventually develop adventive roots directly from the stumps (Figure 3:10) or from the hypocotyl (Figure 3:11). * Another type of abnormal seedling manifests itself by developing the main root and also the primary leaves but is not attached to the surface because of the lack of hypocotyl hairs (1f, 2f), or a lack of geotropic bending of the hypocotyls (3f). All these types of abnormal seedlings usually need a longer period of constant humidity if at least some of them are to get the chance to establish themselves on the substrate. Under natural conditions they will not survive. Seedlings of type 4b-4e are not viable as the seed coat mostly hinders the development of the hypocotyl. Only on rare occasion was epicotyl development observed (4f).

From Tables 2 and 3 it can be seen that certain types of abnormal seedlings occur in high frequency only after the seeds have been stored under certain conditions. A good example is the earlier mentioned case of the storage damaged sallow seed sample (Table 2: -20°C, *without* $CaCl_2$, series 0). In this sample, 87% of the seedlings, although small in size, were at first glance normally developed, but most of them possessed only stumpy 1-2 mm long roots (cf. Figure 3:9). The accidently increased moisture content (19.5%) in these seeds during storage was most probably the reason for this abnormal type of germination. Also the various degree of chlorosis of the cotyledons observed in the present study depends obviously on the specific storage conditions (cf Table 2 and Figure 3:12). A decrease of chlorophyll amount in stored *Salix caprea L.* seeds was earlier determined analytically by Buč (1960). Inhibition of root growth as well as chlorosis of the seedlings can also occur by germination of the seeds in darkness or under low light intensity (Nilsson 1928 by *Salix nigricans*). Other physiological effects of storage were noted by Farmer and Bonner (1967): germinability of *Populus deltoides* seeds, stored for 12 months was reduced very drastically at high temperature in comparison with germination of fresh seeds. In some cases the effect of storage can be discovered only in the later stages of seedling development. Thus Marcet (1957) found that ***Populus tremula*** seedlings raised from stored seeds possessed a lower transplantability. All these examples show how important but also difficult it is to find a correct criterium for "normally germinated seeds" of sallow and aspen.

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* Development of the adventive root from the hypocotyl can be produced artificially even by cutting off the main root.

The transition of the seedlings from stage 6a to 7a can vary in time, depending on the storage conditions of the seeds (cf. series *with* and *without* $CaCl_2$ in Tables 2 and 3). Therefore one cannot exclude the possibility that through some specific storage conditions the epicotyl development can be arrested and thus the seedling development stopped permanently at stage 6a. However, no such case was observed in this study (the experiments were followed for more than 2 months) and therefore seedlings reaching stages 6a or 7a at the end of the germination test (21 days) were accepted as "normally germinated seeds".

Storage experiment

Some degree of drying of the seeds *with* $CaCl_2$ or other hygroscopic agents is necessary for a successful storage of ***Salix*** and ***Populus*** seeds. Sato (1952), for instance, recommends storing the ***Salix*** seeds with an equal or greater amount of adsol (a desiccating agent) than the amount of the stored seeds. However, in another storage experiment, using adsol + K_2S as the drying medium, he demonstrated that a too great amount of the adsol during storage can negatively influence the seed germinability (cf. Table IV, Sato). Moss (1938) also recommends drying the seeds of various poplar and willow to be stored. Using H_2SO_4 of different concentrations as drying agents, he found that the optimum relative air humidity for seed storage appears to be approximately 10 percent for ***Populus balsamifera*** and 15-25 percent for ***Populus tremuloides.*** Below this limit the seeds were damaged. Popcov and Buč (1957) have critically studied the effect of the different degree of drying on seed viability of ***Salix nigricans*** and ***Salix pentandra.*** They came to the conclusion that seeds equilibrated to 10-30 percent of air humidity showed the best storability. As a matter of fact, for long-term storage a 33 percent relative air humidity, i.e., about 7.5 percent m.c. of the seeds, seems to be most preferable (cf. Tables 1, 2 and 3 in Popcov and Buč). The authors make the statement that extreme drying of ***Salix*** seeds over $CaCl_2$ can be detrimental for the seeds viability. Nakajima (1926) also advises caution in the use of concentrated H_2SO_4 or dehydrated $CaCl_2$ for dry storage of ***Salix*** spp. seeds. Polya (1961) in his noteworthy study "Injury by soaking of ***Populus alba*** seeds" found that abnormal seedlings occurred in higher frequency if too dry poplar seeds suddenly came into contact with a moist substrate. He showed that the germinability of the dry seeds could be improved if they were aerated with humid air before putting them on the moist germination substrate. The explanation for this phenomenon is based on the theories of some Russian workers (Genkel and Oknina 1948, Sitnikova 1950 etc). According to them, a sudden rehydration of the desiccated cells in the dried seeds is detrimental for the specific plasmolysis pattern in most ***Salix*** and ***Populus*** species. A cautious prehumification of the overdried seeds makes the cells less susceptible to a moist substrate. Thus, one can assume that the abnormal seedlings are not caused by the storage itself but also by the circumstances occurring when too dry seeds come into sudden contact with a moist substrate.

Summarizing this discussion it is obvious that a drying of seeds for storage purposes is necessary, but only to a limited degree. It is also obvious that various species have different optima for the moisture content of the seeds to be stored.

No doubt, in the present study the $CaCl_2$-drying was too extreme. The amount of hygroscopicum exceeded many times that of the stored seeds and moreover, the glass-tubes with the seeds were continuously exposed to the drying process in the glass-jars (Figure 1). The moisture content of the seeds (*Populus* 2.4% and *Salix* 4.4%) was below the limit of that proposed for optimal storing of willow and poplar seeds by Moss (1938), Popcov and Buč (1957), Zasada and Desmore (1976) etc. Aspen seeds resist drying much better than sallow seeds do (cf. series *with* and *without* $CaCl_2$ at -20°C, in Tables 2 and 3). This result is in good accordance with Buč's storage experiments. According to Buč, the stored seeds of *Populus tremula* preserve their germinability best if dried to as low a moisture content as $< 3.6\%$. For *Salix caprea*, such an extreme drying is harmful (Buč 1960 and Table 1). However, it must be pointed out that the $CaCl_2$ dried aspen seeds produced after 21 days less developed seedlings than the not-dried seeds did - at least in present study (cf. the relation between stages 6a and 7a in Table 3 and Figure 6). Unfortunately, Buč did not report about the quality of seedlings in his experiments.

The moisture contents of the seeds in the series *without* $CaCl_2$ was on the average 8% for sallow and 7.5% for aspen which for the storage of the present material appears to be optimal. This moisture content was reached by predrying of the seeds at a temperature of 35°-40°C when extracting them from catkins and by one days' predrying with $CaCl_2$ in ampoules. It is obvious that when recommending to use hygroscopica for seed preservation in seed storage, the degree of drying must always be indicated. According to Table 1 of Buč (1960), seeds of different species equilibrate themselves during the same period of time and air humidity to different moisture content (cf. also the moisture contents of *Salix* and *Populus* seeds in this paper). Consequently, the final moisture content of the seeds depends also on the length of drying (cf. Faust 1936). Therefore it is better to indicate directly the moisture content to which the seeds should be dried, instead of the air humidity in which the seed drying should be performed.

An atmosphere of desirable humidity for obtaining the right moisture content in seed sample can be produced simply by the so-called equilibration method used by Gustafsson and Simak (1958) for coniferous seeds or by drying the seeds in the presence of different salt solutions (Buč 1960).

Sallow seeds stored *without* cotton showed a higher viability compared to the seeds stored *with* cotton (Table 2). This can be explained due to the better storage regime in the hermetically closed ampoules than in the glass-tubes. Also the one day predrying of the seeds in ampoules could have had a positive effect. The seeds were stored with cotton in order to avoid mechanical damage to the seeds which can possibly occur when removing the cotton. Survival of damaged seeds is short (v. Wettstein 1936, Marcet 1957). On the other hand, storage of seeds with the cotton can also be detrimental if the drying of the seeds is insufficient. The cotton is namely very hygroscopic (cf. Rohmeder 1941, v. Schonborn 1964).

Separation of the seeds from cotton by air stream is a very gentle treatment in comparison with the rather rough rubbing of the seeds over a wire screen with a heavy brush - a method which has been used often in the earlier studies. This study proves that the air stream separation does not cause mechanical damage to the seeds. Moreover, as the seeds *without* cotton can be more easily stored than those *with* cotton, it is recommended to separate the seeds from cotton before putting them in storage.

CONCLUSIONS

On the basis of the results obtained in the present study and also those referred to in other literature, the following recommendations common for storage of sallow and aspen seeds can be made:

1 Fresh collected seeds after the necessary processing should immediately be put into storage.

2 The seeds should be separated from the cotton using methods which do not cause mechanical damage to the seeds.

3 The seeds should be predried to about 7-8% mositure content (on the basis of dry substance) and closed hermetically, e.g., in glass ampoules in which the humidity is constant.

4 The longer storage time that is required, the lower the temperature that should be used (for 44 months storage -20°C was better than ± 0°C or -5°C.

5 Alternative storage methods, using vacuum and freeze drying techniques are attractive but more information is required.

6 For evaluation of the viability of the stored seeds, proper criteria for "normally germinated seed" have to be used.

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STORAGE CONDITIONS FOR WOODY PLANT SEED WITH A HIGH WATER CONTENT

B. Suszka

**Institute of Dendrology,
Kornik, Poland**

ABSTRACT

In the temperate zone, tree seeds have to survive during the winter with a high moisture content and just covered by litter. The survival of seeds in such circumstances depends greatly on the thermal and gaseous conditions. When these conditions can be controlled, germination of seeds can be delayed.

There exists a clear distinction between seeds with a high moisture content which cannot be dried after dispersal below a given relatively high, critical value, and seeds which can be dried to a low critical level. The latter are usually dried and stored at low temperature, but in some circumstances their preservation in a reimbibed condition without loss of viability is possible over a limited period. For both seed types storage at a high hydration level can be combined with their total or partial after-ripening. This is valid, however, only for dormant seeds with fully developed embryos. In this paper, examples are presented on the preservation of highly hydrated seeds of both types from the genera: *Acer*, *Aesculus*, *Fagus*, *Fraxinus*, *Prunus*, *Quercus* and *Taxus*.

RÉSUMÉ

Dans la zone tempérée, les graines des arbres doivent survivre pendant l'hiver alors qu'elles ont un taux d'humdité élevé et qu'elles sont recouvertes par de la litière seulement. Leur survie dans ces conditions dépend beaucoup des conditions thermiques et gazeuses. Quand on peut régulariser ces conditions, il est possible de retarder la germination.

Il existe une distinction nette entre les graines qui possèdent un taux d'humidité élevé et qui ne peuvent pas être déshydratées après leur dispersion jusqu'à une valeur inférieure à une valeur critique donnée, relativement élevée, et les graines qui peuvent être déshydratées jusqu'à une valeur critique faible. En général, ces dernières sont déshydratées et stockées à basse température, mais, dans certaines circonstances, il est possible de les préserver réhumidifiées pendant une courte période, sans perte de viabilité. Il est aussi possible de combiner le stockage de ces deux types de graines, alors qu'elles sont très hydratées, avec leur postmaturation totale ou partielle; toutefois, ceci n'est possible qu'avec des graines dormantes dont l'embryon est complètement développé. La présente communication donne des exemples de préservation, à un taux d'hydration élevé, des deux types de graines, prises chez les genres *Acer*, *Aesculus*, *Fagus*, *Fraxinus*, *Prunus*, *Quercus* et *Taxus*.

INTRODUCTION

In the last 16 years intense work has been done in the Institute of Dendrology in Kornik on storage of seeds of woody-plants in controlled conditions, including species with highly hydrated seeds. We represent the opinion that such investigations should be combined with studies on the optimal conditions for after-ripening of dormant seeds and for germination and seedling emergence of both the non-dormant and the dormant seeds. These conditions can be different for stored seeds, when comparing them with freshly collected non-stored seed material. We present here the optimal variants from our studies. They mainly concern seeds of a high water content, the moisture of which has to be preserved during storage in order to maintain their viability. Also storage conditions were determined for seeds which are usually stored over extended periods when partially dried and were then rehydrated to an unlimited or limited level.

METHODS AND MATERIAL

Seeds of 9 woody species from the north temperate zone were used in the studies. All experiments were performed in controlled thermal conditions. Storage of seeds with a high water content was conducted either on freshly collected material with unchanged or slightly reduced moisture content, or after rehydration of seeds initially dried and dry-stored. Moisture content of the highly hydrated seed was either limited (e.g., *Quercus, Fagus*) or unlimited (e.g., *Fraxinus, Taxus*). Drying of seed, if necessary, was performed only at temperature not exceeding 20°C. As medium for separating seeds from each other during storage, air-dry peat mull or pine sawdust were used. Storage containers differed in capacity from 1 *l* to 100 *l*, depending on the seed dimensions and the available amount for the individual storage variants. Depending on the species and on the experimental design the containers were either covered by perforated lids, closed but not sealed and permitting gaseous exchange, or tightly sealed.

Stratification and seedling emergence tests before and after storage were performed in four 50 seed replications in the laboratory work and in 5 replicates in nursery trials. The laboratory tests were conducted in a moist sand: peat medium with the exception of *Acer saccharinum* seeds, which were germinated on moist filter paper. A radicle of at least 3 mm long was chosen as the criterion for germinated seeds. In the case of some species the growth of epicotyls was also observed (*Aesculus, Quercus*). In seedling emergence tests special replicates were left for the simultaneous observation of the radicle growth.

Chilling of beech seeds without medium at a precise hydration level was based on the weight of the imbibed seeds, calculated previously on the dry matter basis. Losses in moisture content during chilling (i.e., during cold moist storage) were replaced by periodic additions of water until the calculated weight was reached. All water content data were calculated on a fresh weight basis by the oven method (105°C, 24 or 48 hours).

In germination and seedling emergence tests at cyclically alternating temperatures the jars or trays with the stratified or sown seeds were transferred twice daily from the lower temperature for 16 hours to a higher one for the next 8 hours and back. Only for warm-moist stratifications of *Taxus* seeds 24+24 hour cycles were applied. For testing seed viability the cutting test or the indigo carmine embryo staining test has been used.

RESULTS

A. Seeds which cannot be dried without loss of viability below a relatively high critical hydration level.

Seeds belonging to this group can be either non-dormant or dormant. The general pattern for studying such seeds is shown in Fig. 1. Results of our studies on seeds of some species of both types are presented in Figs. 3 and 4.

Non-dormant seeds

Acer saccharinum L. Seeds of silver maple which are dispersed in late Spring are able to germinate immediately. When their initially high moisture content decreases below 50% they lose viability very fast at temperatures normal at that time. These seeds can be stored successfully until the next Spring (Tylkowski 1981), as shown in Table 1.

Table 1. Percentage germinative capacity of seeds of *Acer saccharinum* L. stored in sealed containers. Initial moisture content 49.9%.

Duration of storage	Temperature of storage	Fresh seeds	Seeds aerated at monthly intervals	Seeds never aerated
0 months		86.0		
5 months	1°C		51.5	0.5
	-1°C		76.0	64.0
10 months	1°C		39.5	0.0
	-1°C		55.0	59.0

Best conditions for storage of these seeds with intact samaras are provided by a temperature of -1°C in sealed containers. The seeds may or may not be aereted at monthly intervals. These conditions assure the best height of seedlings.

Quercus robur L. Acorns of the English oak cannot be dried below 40% without loss of viability. Suszka and Tylkowski (1980) have stored them over 4 winters, but viability decreased in that time, especially after the 3rd winter, even in the optimal conditions (Figs. 3 and 7). These conditions are provided by storage in closed but not sealed containers in which the acorns are mixed with air-dry peat or air-dry pine sawdust. These media are necessary to prevent contamination in case of fungal diseases attacking some seeds in the container. When gaseous exchange with the outer air was assured, best results were obtained at -1°C (maximum seedling emergence in laboratory conditions was 72% and in the nursery, 75% after the 3rd winter of storage). At 1°C seeds started to germinate already during storage. At -3°C viability decreased faster than at -1°C.

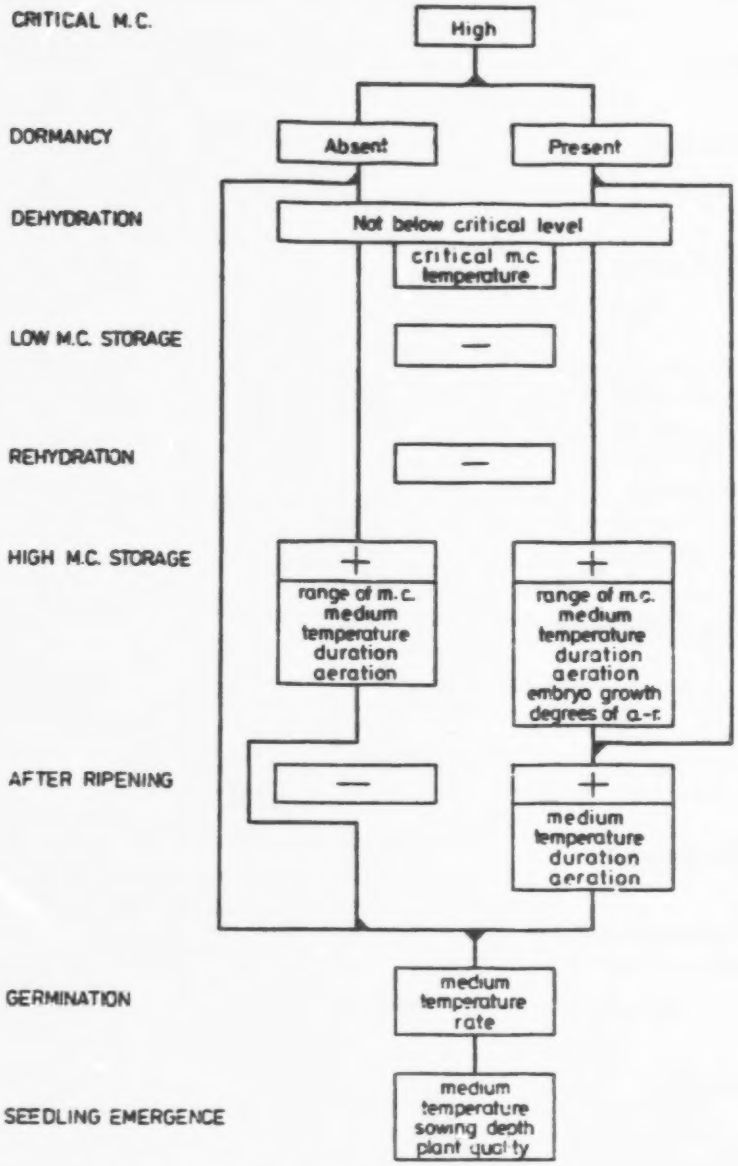

CRITICAL M.C.
High
DORMANCY
Absent
Present
DEHYDRATION
Not below critical level
critical m.c.
temperature
LOW M.C. STORAGE
—
REHYDRATION
—
HIGH M.C. STORAGE
+
range of m.c.
medium
temperature
duration
aeration
+
range of m.c.
medium
temperature
duration
aeration
embryo growth
degrees of a.-r.
AFTER RIPENING
—
+
medium
temperature
duration
aeration
GERMINATION
medium
temperature
rate
SEEDLING EMERGENCE
medium
temperature
sowing depth
plant quality

Figure 1. General design for studying storage conditions of non-dormant and dormant temperate zone woody-plant seeds, which cannot be dried below a relatively high hydration level. Treatments: necessary (+), superfluous or impossible (-). M.C. = moisture content; a.-r. = after-ripening.

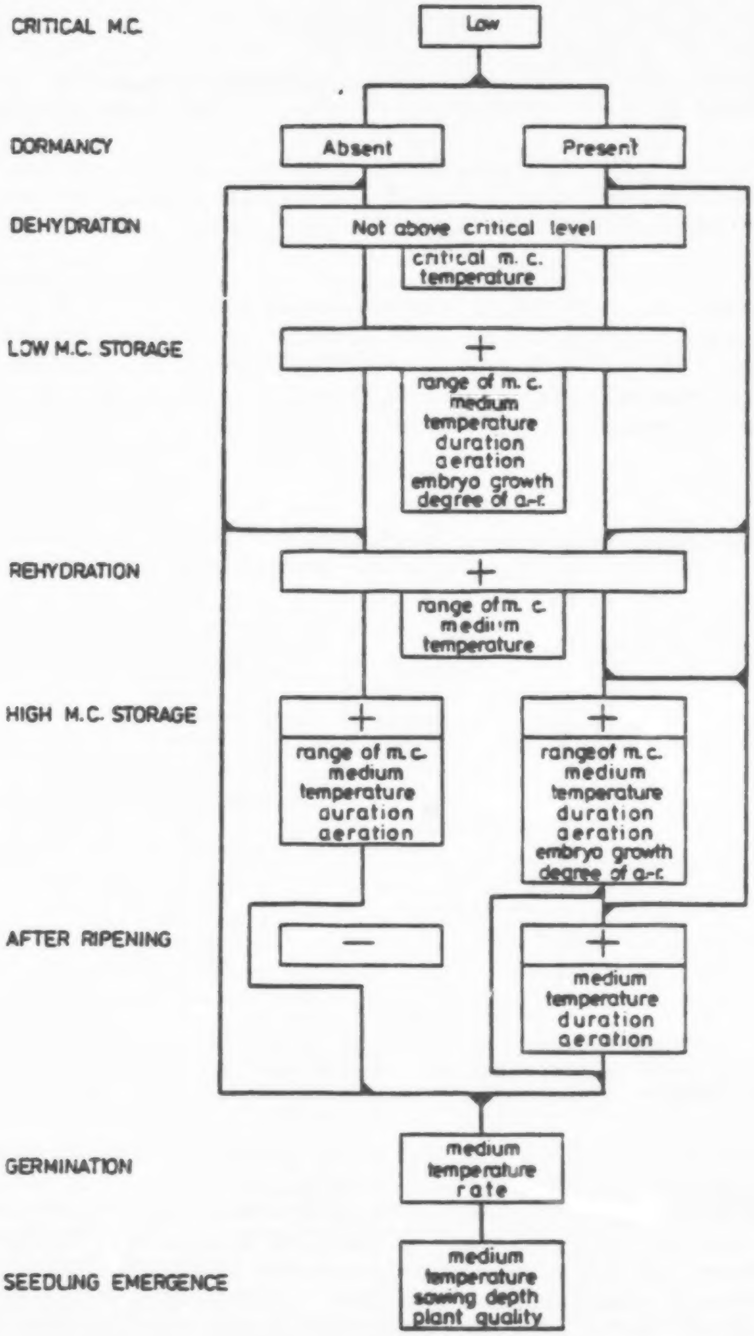

CRITICAL M.C.
Low
DORMANCY
Absent
Present
DEHYDRATION
Not above critical level
critical m. c.
temperature
LOW M.C. STORAGE
+
range of m. c.
medium
temperature
duration
aeration
embryo growth
degree of a.-r.
REHYDRATION
+
range of m. c.
medium
temperature
HIGH M.C. STORAGE
+
range of m. c.
medium
temperature
duration
aeration
+
range of m. c.
medium
temperature
duration
aeration
embryo growth
degree of a.-r.
AFTER RIPENING
−
+
medium
temperature
duration
aeration
GERMINATION
medium
temperature
rate
SEEDLING EMERGENCE
medium
temperature
sowing depth
plant quality

Figure 2. General design for studying storage conditions of non-dormant and dormant temperate zone woody plant seeds, which sustain a low hydration level and are usually dry-stored. Design includes possibility of storage at elevated hydration levels. Treatments: necessary (+), superfluous or impossible (-). M.C. = moisture content; a.-r. = after-ripening.

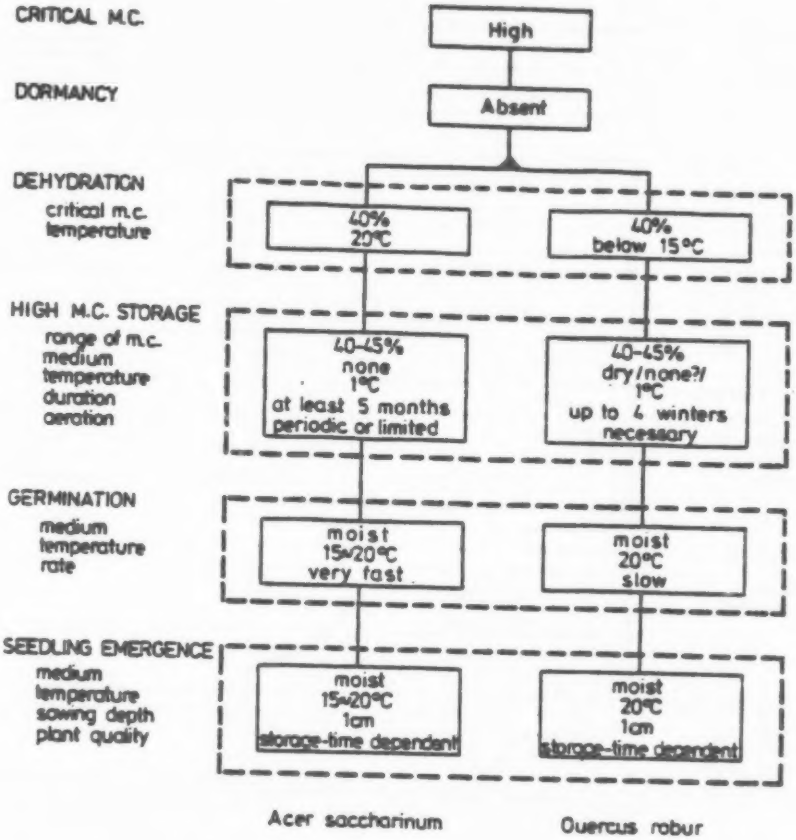

CRITICAL M.C.
High
DORMANCY
Absent
DEHYDRATION
critical m.c.
temperature
40%
20°C
40%
below 15°C
HIGH M.C. STORAGE
range of m.c.
medium
temperature
duration
aeration
40-45%
none
1°C
at least 5 months
periodic or limited
40-45%
dry/none?/
1°C
up to 4 winters
necessary
GERMINATION
medium
temperature
rate
moist
15-20°C
very fast
moist
20°C
slow
SEEDLING EMERGENCE
medium
temperature
sowing depth
plant quality
moist
15-20°C
1cm
storage-time dependent
moist
20°C
1cm
storage-time dependent
Acer saccharinum
Quercus robur

Figure 3. Examples of storage at a high moisture content of non-dormant seeds, which cannot be dried below a high critical hydration level. M.C. = moisture content; a.-r. = after-ripening.

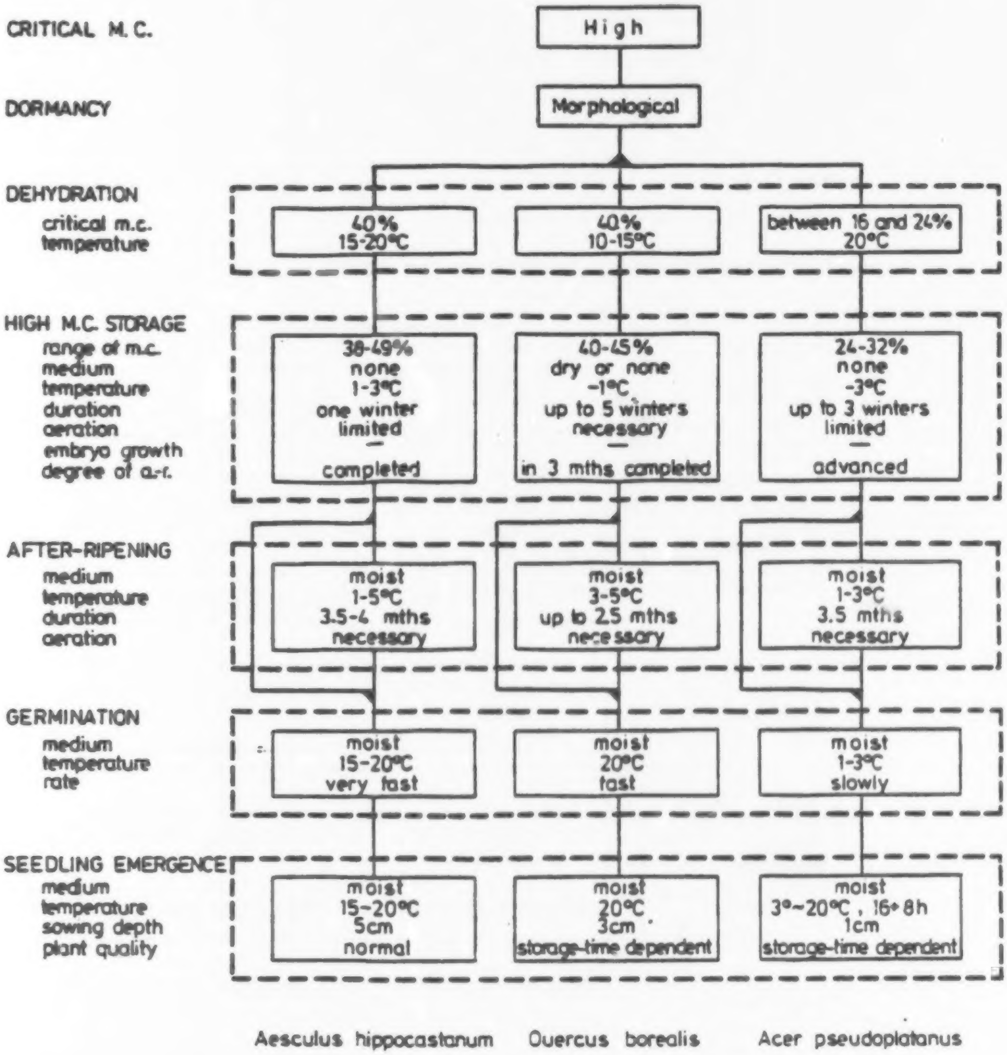

CRITICAL M.C.
High
DORMANCY
Morphological
DEHYDRATION
critical m.c.
temperature
40%
15-20°C
40%
10-15°C
between 16 and 24%
20°C
HIGH M.C. STORAGE
range of m.c.
medium
temperature
duration
aeration
embryo growth
degree of a.-r.
38-49%
none
1-3°C
one winter
limited
—
completed
40-45%
dry or none
-1°C
up to 5 winters
necessary
—
in 3 mths completed
24-32%
none
-3°C
up to 3 winters
limited
—
advanced
AFTER-RIPENING
medium
temperature
duration
aeration
moist
1-5°C
3.5-4 mths
necessary
moist
3-5°C
up to 2.5 mths
necessary
moist
1-3°C
3.5 mths
necessary
GERMINATION
medium
temperature
rate
moist
15-20°C
very fast
moist
20°C
fast
moist
1-3°C
slowly
SEEDLING EMERGENCE
medium
temperature
sowing depth
plant quality
moist
15-20°C
5cm
normal
moist
20°C
3cm
storage-time dependent
moist
3°-20°C, 16+8h
1cm
storage-time dependent
Aesculus hippocastanum
Quercus borealis
Acer pseudoplatanus

Figure 4. Examples of storage at a high moisture content of dormant seeds, which cannot be dried below a high critical hydration level. M.C. = moisture content; a.-r. = after-ripening.

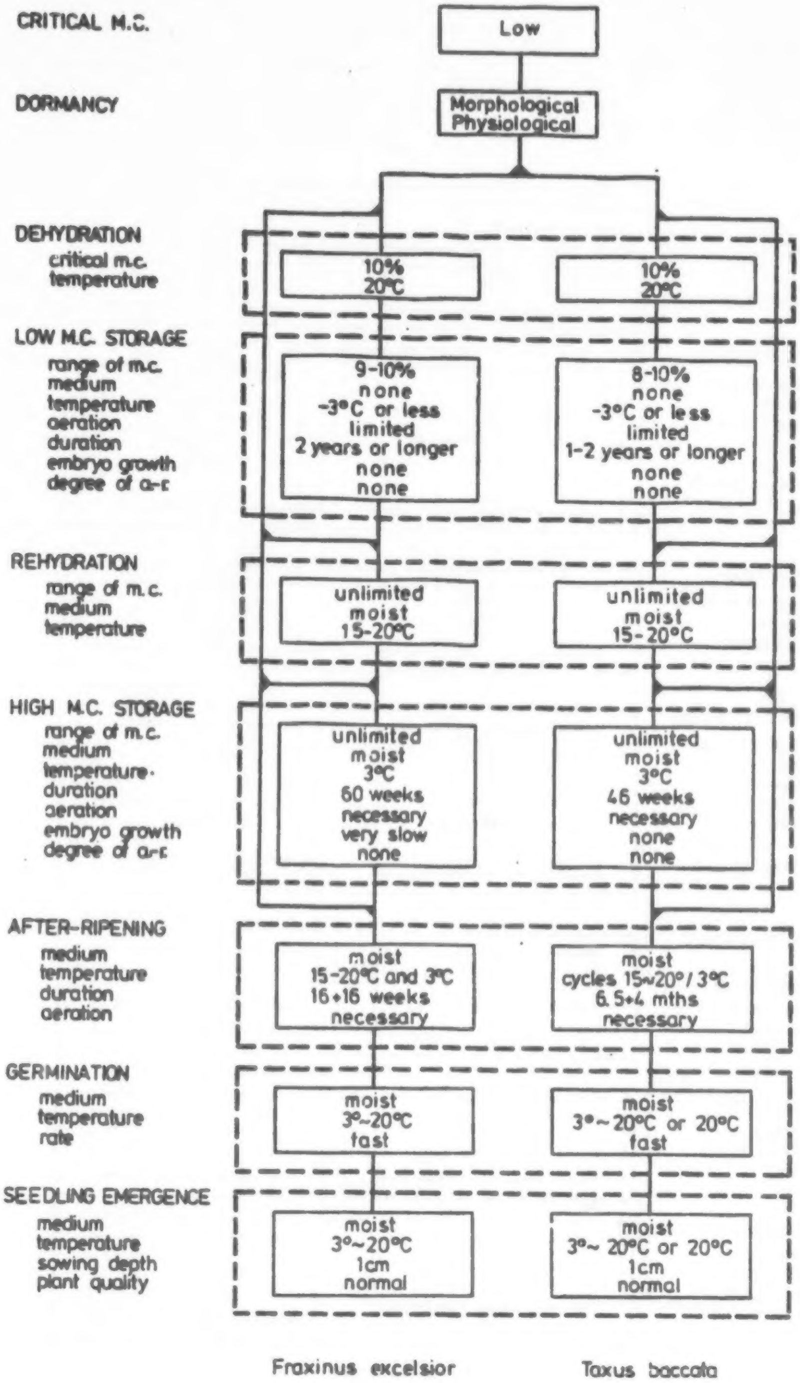

CRITICAL M.C.
Low
DORMANCY
Morphological
Physiological
DEHYDRATION
critical m.c.
temperature
10%
20°C
10%
20°C
LOW M.C. STORAGE
range of m.c.
medium
temperature
aeration
duration
embryo growth
degree of a-r
9-10%
none
-3°C or less
limited
2 years or longer
none
none
8-10%
none
-3°C or less
limited
1-2 years or longer
none
none
REHYDRATION
range of m.c.
medium
temperature
unlimited
moist
15-20°C
unlimited
moist
15-20°C
HIGH M.C. STORAGE
range of m.c.
medium
temperature
duration
aeration
embryo growth
degree of a-r
unlimited
moist
3°C
60 weeks
necessary
very slow
none
unlimited
moist
3°C
46 weeks
necessary
none
none
AFTER-RIPENING
medium
temperature
duration
aeration
moist
15-20°C and 3°C
16+16 weeks
necessary
moist
cycles 15~20°/3°C
6.5+4 mths
necessary
GERMINATION
medium
temperature
rate
moist
3°~20°C
fast
moist
3°~20°C or 20°C
fast
SEEDLING EMERGENCE
medium
temperature
sowing depth
plant quality
moist
3°~20°C
1cm
normal
moist
3°~20°C or 20°C
1cm
normal
Fraxinus excelsior
Taxus baccata

Figure 5. Examples demonstrating the possibility of storage at a high moisture content of dormant seeds with underdeveloped embryos which are usually dry-stored. M.C. = moisture content; a.-r. = after-ripening.

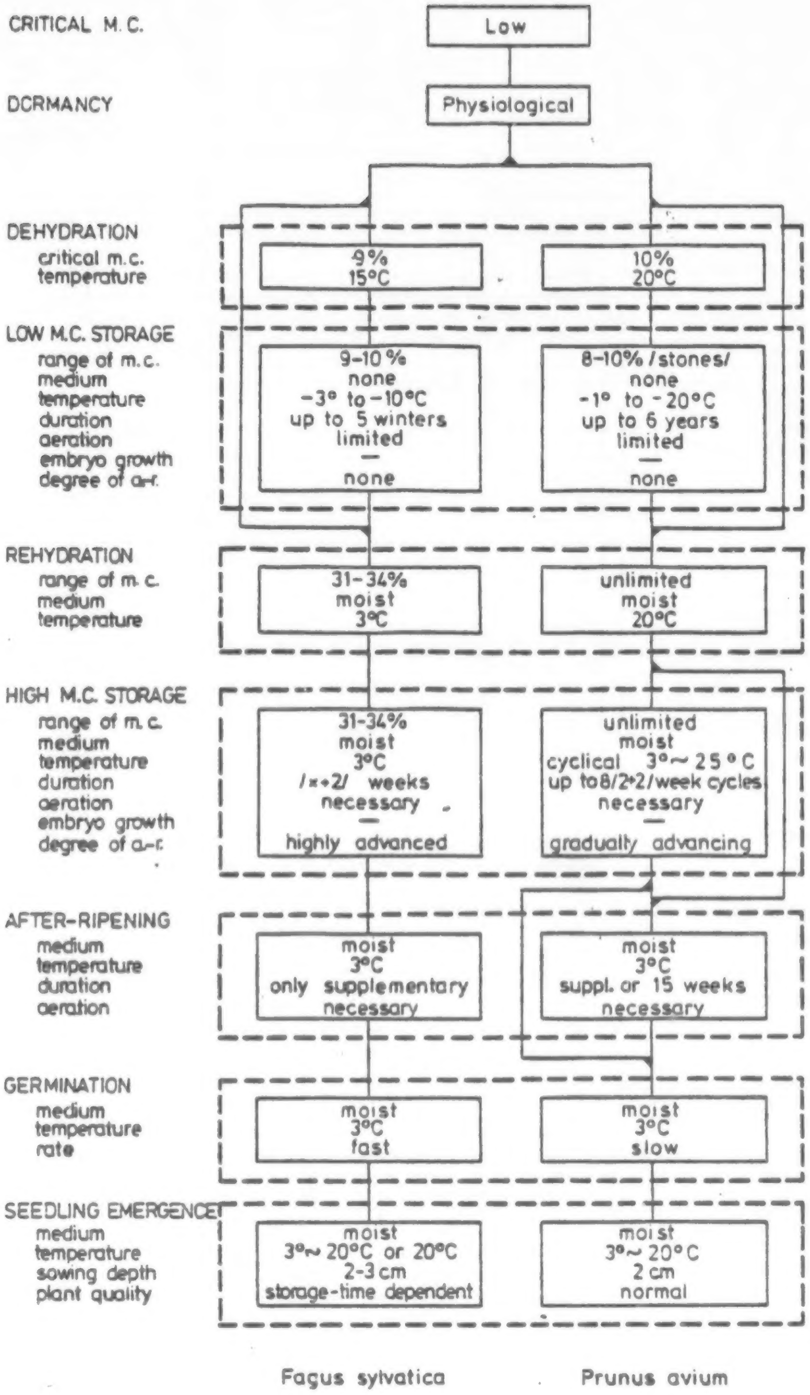
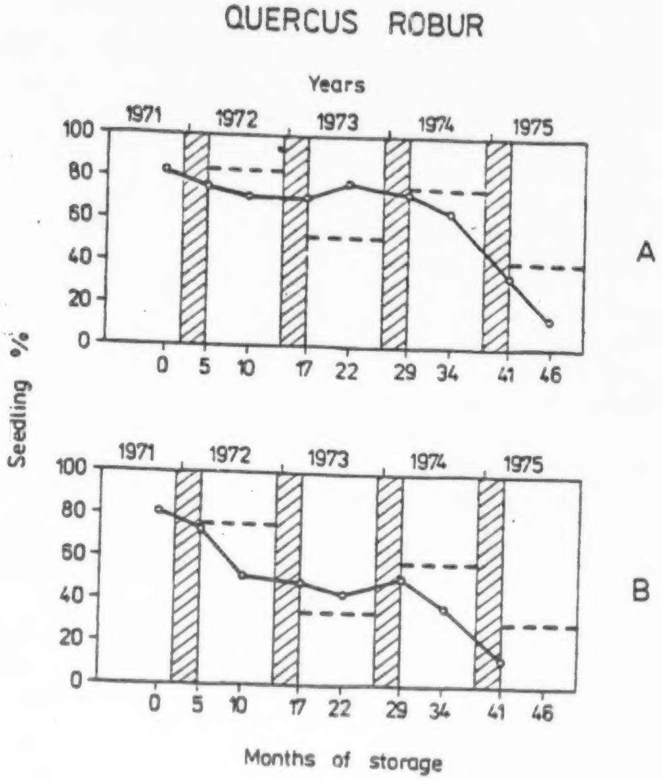

CRITICAL M.C.
Low
DORMANCY
Physiological
DEHYDRATION
critical m.c.
temperature
9%
15°C
10%
20°C
LOW M.C. STORAGE
range of m.c.
medium
temperature
duration
aeration
embryo growth
degree of a-r.
9-10 %
none
-3° to -10°C
up to 5 winters
limited
—
none
8-10% /stones/
none
-1° to -20°C
up to 6 years
limited
—
none
REHYDRATION
range of m. c.
medium
temperature
31-34%
moist
3°C
unlimited
moist
20°C
HIGH M.C. STORAGE
range of m. c.
medium
temperature
duration
aeration
embryo growth
degree of a.-r.
31-34%
moist
3°C
/x+2/ weeks
necessary
—
highly advanced
unlimited
moist
cyclical 3°~ 25 °C
up to 8/2+2/week cycles
necessary
—
gradually advancing
AFTER-RIPENING
medium
temperature
duration
aeration
moist
3°C
only supplementary
necessary
moist
3°C
suppl. or 15 weeks
necessary
GERMINATION
medium
temperature
rate
moist
3°C
fast
moist
3°C
slow
SEEDLING EMERGENCE
medium
temperature
sowing depth
plant quality
moist
3°~ 20°C or 20°C
2-3 cm
storage-time dependent
moist
3°~ 20°C
2 cm
normal
Fagus sylvatica
Prunus avium

Figure 6. Examples demonstrating the possibility of storage at a high moisture content of dormant seeds with fully developed embryos which are usually dry-stored. M.C. = moisture content; a.-r. = after-ripening.


QUERCUS ROBUR

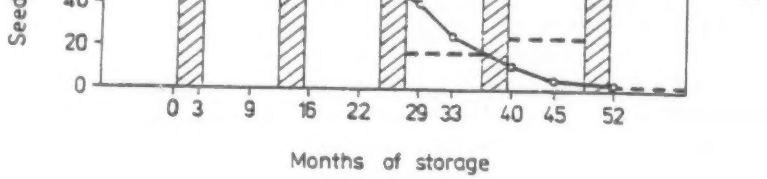

QUERCUS BOREALIS

Figure 7. Long-term storage of acorns of *Quercus robur* L. and of ***Q. borealis*** Michx. in closed but not sealed containers at -1°C and at an initial moisture content of 45.6% and 44.8% respectively, mixed with air-dry peat (A) or air-dry pine sawdust (B). Solid line: changes of seedling emergence at 20°C; broken line: seedling percent in the nursery after Spring outdoor sowings.

Dormant seeds

Aesculus hippocastanum L. Freshly collected horsechestnut seeds need about 110 days of stratification at 3°C and after that they are able to germinate in a broad range of temperatures from 1° to 20°C, but epicotyls grow intensively only in the upper part of this range. When the initially high moisture content of 49% was decreased to 38%, about 20% of seeds have lost viability. The undried seeds have retained germinability at the 100% level (radicle and epicotyl) when stored at 1°C in jars sealed with a double polyethylene sheet. Storage temperatures of 3° and 5°C assured also excellent results, the optimal germination temperature for so stored seeds being 15°C. At this temperature all viable seeds germinated and their epicotyls grew within the first 15 days (Figs. 4 and 8). In field conditions 75% of seeds produced seedlings after storage at 1-5°C in the above described way over one winter (Suszka 1966a). Other investigators (Widmoyer and Moore 1968) reported the retaining of 25% viable seeds after 15 months of sealed storage of fresh seeds at -1.1°C.

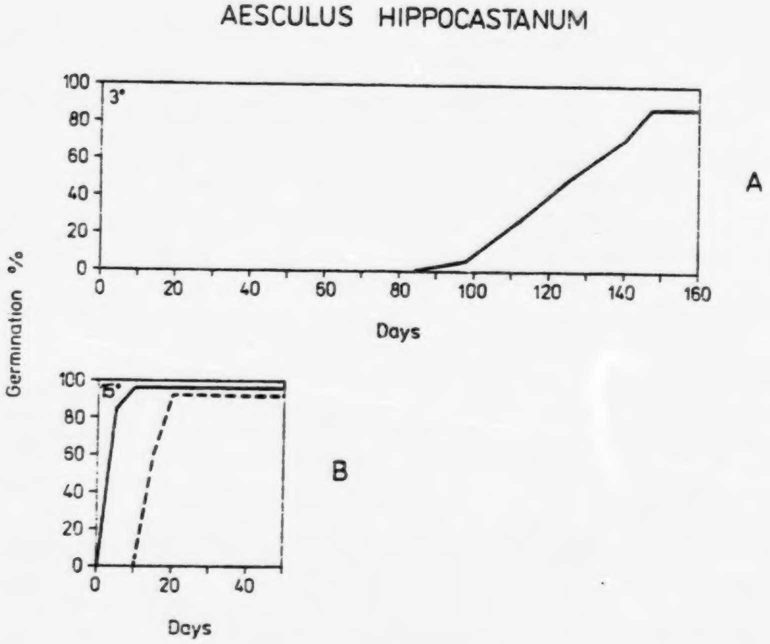


Figure 8. Course of germination (solid line) and epicotyl growth (broken line) of *Aesculus hippocastanum* L. seeds stratified at 3°C immediately after dispersal (A), or after 187 days of storage at 3°C in containers sealed with a double sheet of polyethylene, when sown at 15°C (B). Initial moisture content before storage was 48.6%.

Quercus borealis Michx. When acorns of the Northern red oak are freshly collected, they start to germinate early at 20°C but sluggishly and at a low percentage. At 5°C germination does not start before 80 days. When stratified at 5°C for 70 days and then transferred to 20°C they initiate germination immediately and within the next 50 days all viable seeds have growing roots and epicotyls.

These acorns can be stored like the non-dormant acorns of the English oak, and even over 5 winters (Suszka and Tylkowski 1976) at -1°C, when mixed with dry peat. In the case of dry sawdust or when no medium is used, the possible storage period does not exceed 4 winters. Germinability decreases in these conditions with the decrease being most pronounced after the 2nd winter. In the second Spring after collection, germination and seedling emergence of about 50% can still be assured in the described above conditions (Figs. 4 and 7).

Acer pseudoplatanus L. Seeds of the sycamore maple with intact samaras are deep-dormant after dispersal and need about 15 weeks of stratification at 1-3°C before germination starts. Their moisture content cannot drop below 16-24% without loss of viability. Storage is possible only when a moisture content of 24-32% is assured. Such samaras can be held only in sealed containers, protecting them from further desiccation. But, to minimize the metabolic activity in the seed tissues, a storage temperature of -3°C has to be maintained (Figs. 4 and 9). This temperature is better than -1°C or temperatures just above 0°C. In these conditions germinability is retained, though gradually decreasing, even over 3 winters and the period of a further cold stratification is shortened to 6-8 weeks only (Suszka 1979).

B. Seeds which can be dried without loss of viability to a relatively low hydration level.

Included in this group are seeds which were previously dried to a low moisture content (below 10%) and stored usually over some years. However, they can also be stored for much shorter periods when they are reimbibed at a low or alternating temperature, under aeration. Storage of non-dormant seeds of this type has not been studied in our laboratory, but sufficient experience was gathered for seeds exhibiting dormancy when ripe. Moist storage of seeds of some species can be combined with their after-ripening, when access of water to the seeds is not interrupted. The general pattern for studying these seeds is presented in Fig. 2, and the detailed procedures in Figs. 5 and 6.

Dormant seeds with underdeveloped embryos

Fraxinus excelsior L. Seeds of the European ash should be stratified first for 16 weeks at 15-20°C to enable the underdeveloped embryos to grow. This should be followed by cold stratification at 3°C for 16 weeks, which is sufficient for after-ripening. So pretreated seeds germinate quickly at a daily alternating temperatures of 3° to 20°C (Suszka 1978). When held in the imbibed condition at 3°C for 60 weeks, no more than 2-3% of seeds germinate (Suszka, unpublished), all other seeds remaining viable. In this period the growth of embryos is insignificant. We can consider such a treatment as cold-moist storage (Fig. 5).

Taxus baccata L. Seeds of the English yew need a long period (6 months) of after-ripening at alternating temperatures for the promotion of growth of embryos and followed by a cold period to break their dormancy (Devillez 1976). After that they need a new period with daily alternating temperatures for germination and seedling emergence (Suszka, unpublished). We have found that the imbibed seeds can be stored at 1-3°C for 46 weeks in a moist medium. In that period embryo growth cannot occur because of the low temperature, and the undergrown embryos do not benefit from chilling. However, viability of seeds and of the enclosed embryos is fully retained and therefore we can consider cold stratification of yew seeds as an example of cold-moist storage.

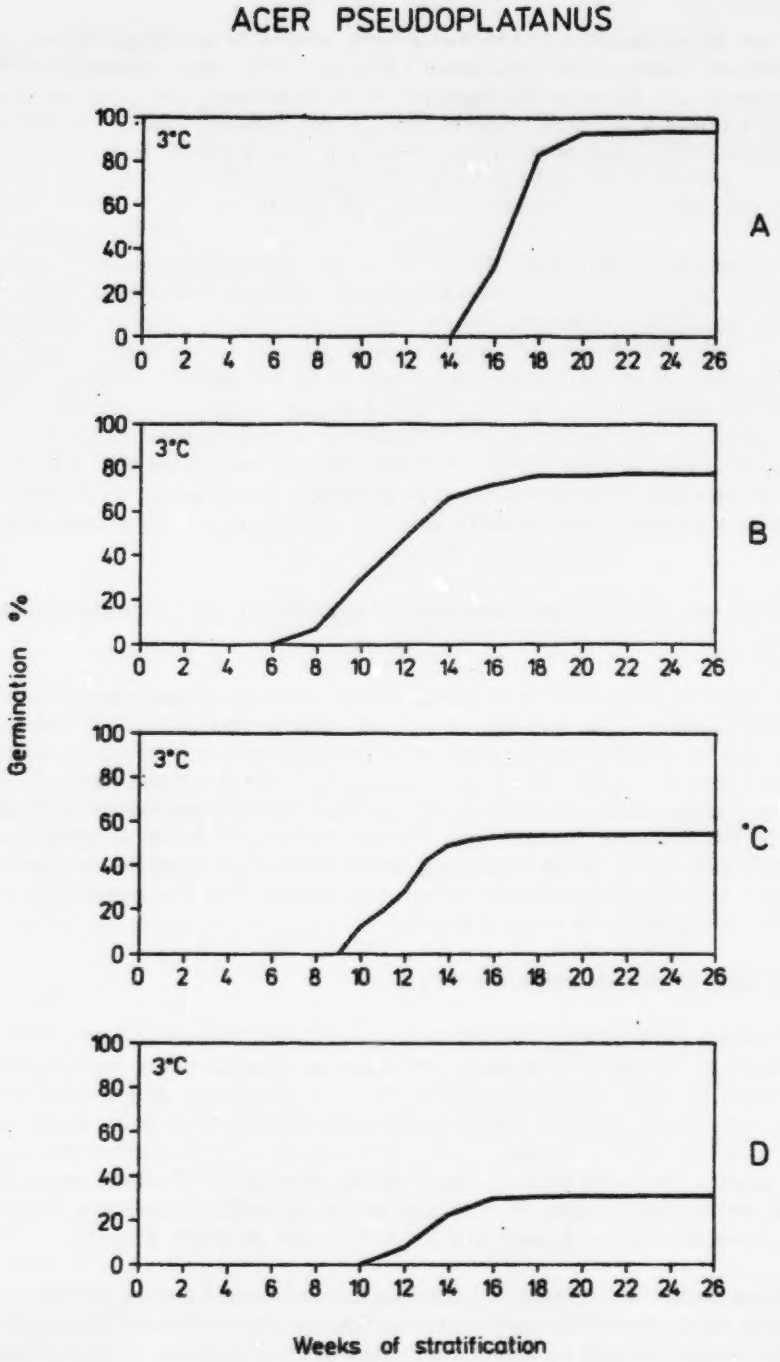


Figure 9. Course of germination of *Acer pseudoplatanus* L. seeds: during stratification at 3°C shortly after dispersal (A) or after storage at -3°C in sealed bottles over one (B), two (C) and three (D) winters. Initial moisture content of samaras was 24%.

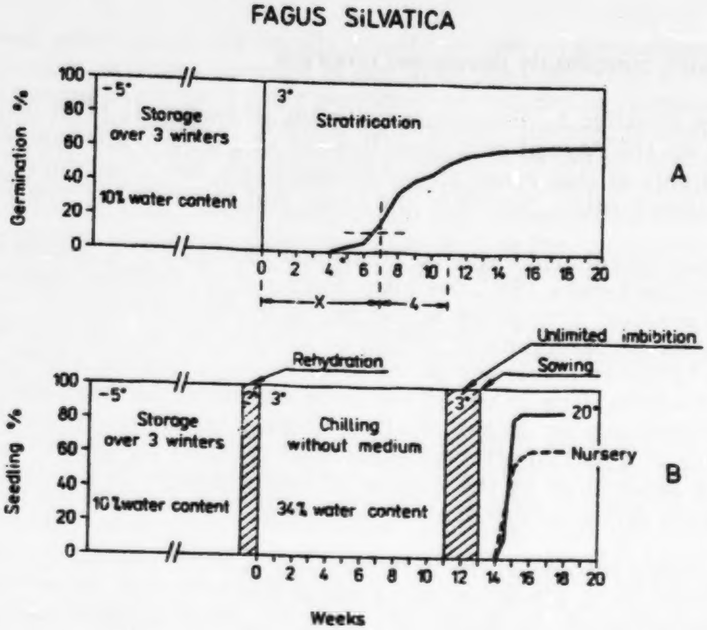


Figure 10. Course of germination at 3°C of *Fagus silvatica* L. seeds after dry storage at -5°C over 3 winters (A) and seedling emergence at 20°C and in the nursery after Spring sowing; seeds were chilled without medium (cold moist storage) for (x+4) weeks at 3°C and pregerminated at the same temperature for 2 weeks (B).

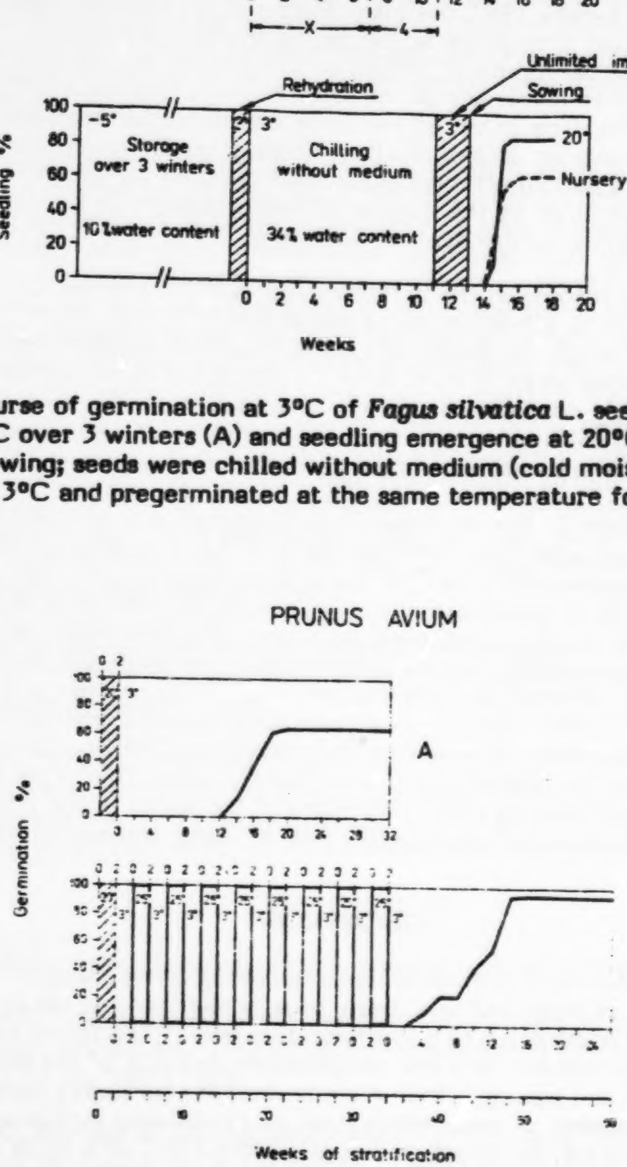


Figure 11. Course of germination of *Prunus avium* L. seeds during a warm (20°C) and followed by a cold (3°C) stratification (A), and during a similar stratification (moist storage) when cyclically interrupted every 2 weeks by an inductive treatment (25°C for 2 weeks) repeated eight times (B).

Dormant seeds with completely developed embryos

Fagus silvatica L. Beechnuts collected immediately after dispersal need a period of 40-100 days of cold stratification at 1-5°C to start germination, which is possible only at that range of low temperatures for 50-60 days (Suszka 1966b). At this temperature growth of the hypo- and epicotyls is completely arrested.

We have developed a special treatment (Suszka 1979, Suszka and Zieta 1976, Suszka and Kluczynska 1980, see also: Perrin et al. 1978) to make a fast germination and seedling emergence at various temperatures possible. This treatment includes rehydration of beechnuts at 3°C up to 31-34% and maintaining their storage at this precisely hydrated levels and at the same temperature for a period of "x" plus the next 2-4 weeks. The value "x" has to be estimated for each seed lot separately, because of the varying degree of after-ripening for different provenances and the possibility of a partial after-ripening when lying on the ground before collection. This "x" period is determined by the number of weeks needed for germination of 10% of viable seeds during a normal stratification at 3°C.

After cold storage of the imbibed seeds without any medium at the restricted hydration level, a free access of water during chilling at 3°C triggers their germination, although the length of radicles does not exceed 2 mm in that period. Such seeds, when sown at 20°C or at a cyclically alternating temperature of 3° to 20°C or in field conditions in Spring, germinate fast and seedlings emerge without delay. During chilling without medium, dormancy of beech seeds disappears. This special kind of storage enables a quick germination (Figs. 6 and 10).

Prunus avium L. Mazzard seeds can be stored in a dry condition for some years. They have to be stratified first at 20°C for 2 weeks, and afterwards at 3°C for about 15 weeks, until the first seeds start to germinate. At this low temperature the course of germination is slow, but placing the seeds in warmer conditions (e.g., at 20° or 25°C) induces a secondary dormancy (Suszka 1967, 1973). We have found that germination can be delayed when the seeds are subject to rehydration at a cyclically alternating temperature, each cycle consisting of 2 weeks at 3°C and 2 weeks at 25°C. In such conditions seeds start to germinate not earlier than after 8 cycles i.e., after 32 weeks of pretreatment. Germination starts without any delay and germinative capacity can be extremely high. This triple effect is provided by the cyclically repeated inductions of dormancy in the chilled seeds. This treatment can be regarded as a special case of moist storage of seeds (Figs. 6 and 11).

DISCUSSION

We should clearly distinguish between seeds whose initially high moisture content can never be reduced below a given, usually high critical value, from seeds which are capable of being dried to a low hydration level. The latter can be stored easily in sealed containers and at a low temperature, because of the nearly total arrest of metabolism and ageing. When storage methods for highly hydrated seeds have to be found, a number of parameters must be established including: the permissible range of moisture content, temperature (usually around 0°C or a little below it), the necessity for a medium separating the seeds during storage, necessity and scale of gaseous exchange and aeration. When the seeds are dormant, the degree of dormancy still present after storage as well as the type of treatment required for its total elimination have to be recognized. Also conditions for germination and elongation of hypo- and epicotyls (i.e., for seedling emergence) should be found. The general designs for such studies are presented in Fig. 1.

Most seeds which are usually dry-stored can retain viability for long or very long periods, whether dormant or not when predried. There exists however, a possibility to store them for shorter periods in an imbibed or reimbibed condition at low temperature and under air access. During such storage, growth of the underdeveloped embryos (when such embryos are present) is inhibited totally or strongly. It should be remembered that dormancy can disappear totally or partially only in the fully grown embryos. In any case, conditions necessary for the termination of the after-ripening processes (if necessary) as well as the conditions for germination and hypo- and epicotyl growth have to be found. Each species has to be studied separately and generalizations are not possible. In case of big differences in the depth of dormancy still present between various lots of seeds of the same species, the variability has also to be taken into consideration when methods are modified for general application (Fig. 2).

We should bear in mind that moist storage of seeds occurs very commonly in natural environmental conditions i.e., in the soil. High temperatures in Spring can result in an induction of secondary dormancy or even in multiple inductions of dormancy from year to year. This enables seeds even from one specimen and from a single crop to germinate and produce seedlings in several consecutive years. This seems to be an important adaptation, widening the period of seedling production from one to many vegetative periods which is quite opposite to what the nurserymen need. Dry storage in natural conditions of the temperate zone is rather usual, and even seeds which remain over the winter in the fruits on trees or shrubs undergo changes in their moisture content. The most common phenomenon is moist storage in the soil, where it serves as a seed bank with a multitude of possible combinations of storage conditions, depending on the depth of the seed burial, the effectiveness of insulation from the thermal effects of sunshine and low temperature, the possibility of access of water to the seeds and on the composition of the soil atmosphere. This fact is well known, but so far mainly seeds of herbaceous species have been studied in this respect (Barton 1961).

The examples presented in this paper illustrate our attempts to imitate and to simplify the conditions present in the natural soil environment of seeds of woody plants in the temperate zone.

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LONG TERM STORAGE OF BEECHNUTS: RESULTS OF LARGE SCALE TRIALS

C. MULLER

Station d'Amélioration des Arbres Forestiers
I.N.R.A. CNRF (Nancy) Champenoux 54280 SEICHAMPS - France -

M. BONNET-MASIMBERT

Station d'Amélioration des Arbres Forestiers
I.N.R.A. CRF (Orléans) Ardon 45160 OLIVET - France -

ABSTRACT

Based on the main results of laboratory experiments on storage of beechnuts by drying the seeds to a moisture content of 8-9% at room temperature and storing them in hermetically sealed containers between -5°C and -10°C, it was possible to successfully store 17 tons of beechnuts from 51 different sources for 4 to 6 years. This storage method has now been adopted for operational practice in France.

RÉSUMÉ

D'après les principaux résultats d'expériences en laboratoire au cours desquelles on a déshydraté des faînes jusqu'à un taux d'humidité de 8 à 9% pour ensuite les stocker dans des contenants hermétiques, à -5°C et -10°C, on a réussi à stocker 17 tonnes de faînes provenant de 51 sources pendant 4 à 6 ans. Cette technique de stockage est maintenant utilisée couramment en France.

INTRODUCTION

For a long time, regeneration of the French beech forests was essentially a natural one. But, because of very episodic beech masts and frequent failure in natural regeneration, it became more and more necessary to use artificial regeneration either as a complement to the natural one, or as a substitution to it. This required intensified research work to elaborate methods making possible a successful long-term storage of beech seeds after a mast year. In this way, nurseries could be supplied with stored seeds for years with bad crops.

This report deals only with long term storage for which trials on a large scale have been made by the French "Office National des Forêts" (O.N.F.).

Nyholm (1960), Buszewicz (1964) and Schoñborn (1964) were among the first to try to store beechnuts. In some cases these authors succeeded in storing them in good conditions for 2 to 3 years. But it is especially since 1966-1967 that studies on the long-term storage of beech seeds were started, mainly in Poland by Suszka, and in France by Bonnet-Masimbert and Muller.

Suszka (1974) succeeded in maintaining for 5 winters a high germinative capacity of beechnuts dried up to 9-10% relative moisture content and stored in sealed containers at -10°C. Small scale similar experiments were simultaneously conducted in France since 1967 and was followed by new trials on lots with more than 10 kg (1972-1974). The first large scale experiments were carried out in 1974 and 1976 involving 17 tons of beechnuts, corresponding to 51 individual lots, stored by O.N.F. In this paper we briefly report the results of these trials.

SMALL SCALE LABORATORY TRIALS ON EXPERIMENTAL LOTS

Several tests were carried out in our tree seed laboratory in 1967, 1972 and 1974 to study the influence on storage of such factors as method of drying, moisture content, temperature of storage, and type of conditioning. After the 1967 trials, it appeared that the best conditions to maintain a good level of germination during 4 years were: moisture content near 8-9% after drying at room temperature; hermetically sealed containers; and storage at a temperature below 0°C (Bonnet-Masimbert et Muller 1973). This was confirmed by experiments in 1972 and 1974 by Bonnet-Masimbert and Muller (1975), the results of which appear on Table 1. After 7 and 5 years respectively of storage, either at -5°C or at -15°C and whatever the method of drying at room temperature may have been, a decline in germinative capacity could not be observed.

Comparing the initial with the 1979 values of germination in Table 1, we can observe an increase of percentage. The differences in germination are due to the harmful effects of a 24-hour soaking in water before the germination test. We demonstrated (Muller 1977) that it was a dangerous practice. Thus, in 1979, germination tests were performed without the preliminary soaking. The results obtained confirmed the earlier findings: the necessity of drying the beechnuts to 8-9% moisture content (fresh weight basis) by ventilation at room temperature (18-20°C) without heating and then storing them in hermetically sealed containers at between -5°C and -10°C.

This method of storage should be coupled with a treatment against fungi contaminating the stored seed material. We frequently observe at the cropping time, a strong attack of beechnuts by *Rhizoctonia solani*, a telluric fungus which alters the quality of the seeds before and after storage (Le Tacon *et al.* 1976). Chemical treatment (PCNB, active compound) employed before storage, during stratification or before sowing gave satisfactory results. We tried to eliminate *Rhizoctonia* using a thermotherapy treatment (24 hours at 37°C) (Perrin *et al.* 1978). This treatment, however, is not always compatible with storage and further studies are still needed.

Table 1. Long term storage of beechnuts in 30 litre containers- Results after 5 and 7 years of storage (Laboratory trials).

Lot number	Origin	Year of crop	Type of drying	Storage		Germination percentage (2)	
				Moisture content % (1)	Temperature (°C)	Initial	1979
72074	Lyons la Forêt	1972	Air (3)	9,0	- 5	79	86
				9,0	- 15	79	89
			Seed (4) drier	7,7	- 5	83	78
				7,7	- 15	81	91
74084	Compiègne	1974	"	9,0	- 5	85	85
				9,0	- 15	85	83
74085	Joinville	1974	"	8,2	- 5	80	82
				8,2	- 15	80	76

(1) The moisture content is determined on the fresh weight basis.
(2) Germination at 4°C in stratification conditions, during 4 months.
(3) Air drying for 6 days at room temperature (18-20°C).
(4) Ventilated seed drier (without heating) during 18 hours.

LARGE SCALE EXPERIMENT

Our methodology was employed by the O.N.F. for storage of more than 17 tons of seeds from the 1974 and 1976 beechmasts. The results are presented in Tables 2 and 3. For most seed lots it is clear that a very high level of germination is maintained, even after 4 to 6 years of storage. The experiment will be continued with those seed lots that are not yet used for plantation work by the O.N.F. Nevertheless we can consider these results as being very encouraging for practical application.

CONCLUSION

It has been several years since research laboratories, especially in Poland and in France, have been able to successfully store beechnuts for their own needs. Our results have demonstrated that this technique can now be applied in practice.

The problem of the long-term storage of beechnuts can be regarded now as solved. From an idea by Suszka (1975) we are now working on the possibility to incorporate the treatment for breaking dormancy in the storage process. The aim of such a procedure would be to provide foresters with stored dry beechnuts which are ready to germinate after sowing. Our preliminary results (not presented here) indicate that it should be also possible to store after-ripened beechnuts for at least 15 months.

Table 2. Large scale storage of beechnuts by O.N.F. -1974 beechmast- Results after 6 years of storage at -5°C.

Lot number	Weight (kg)	Moisture content % f.w.	Germination percentage			
			1974[1]	1976	1978	1980
74.01	390	8,4	63	68		
74.02	170	8,5	68	66		
74.03	885	9,5	65	71		
74.04	23		51	66		
74.05	157	9,7	64	65		
74.06	554	9,9	83	77	86	75
74.07	891	8,3	81	77	86	82
74.08	48	8,5	64	55		
74.09	35	9,2	76	80		
74.10	3	7,9	87		89	88
74.11	101	8,3	57			
74.12	36	8,8	88			
74.13	47	8,0	91		92	91
74.14	19	8,6	81			
74.15	24	8,5	73	64		
74.16	32	8,7	79	80	88	87
74.17	24	8,4	86	80	84	80
74.18	87	8,4	61	70	79	85
74.19	38	9,1	84	86		
74.20	177	9,6	63		71	73
Total	3 741					

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[1] The germination method changed after 1976. That would explain some increase of germination percentage between 1974 and other years. As an example, the lot No. 74.18 might be very sensitive to the preliminary soaking.

Table 3. Large scale storage of beechnuts by O.N.F. -1976 beechmast. Results after 4 years of storage at -5°C.

Lot number	Weight (kg)	Moisture content % f.w.	Germination percentage 1976	1978	1979	1980
76.01	2347	8,2	80	81	86	83
76.02	637	8,8	80	80	78	
76.03	1499	8,3	84	78	84	
76.04	371	8,8	88	86	83	
76.05	95	9,4	73	75		
76.06	915	8,6	84		91	
76.07	608	8,8	79	86	86	
76.08	584	8,6	79	84	76	
76.09	127	8,9	91	87	78	
76.10	65	7,5	91	97		
76.11	459	7,6	76		80	
76.12	283	6,8	81	90	74	
76.13	277	6,7	68			
76.14	124	7,1	52	52		
76.15	1222	8,3	94	92	94	94
76.16	1445	8,6	71		78	
76.17	245	9,2	66	68	59	
76.18	54	8,8	73			
76.19	135	8,1	88		75	
76.20	204	9,2	66	72		
76.21	301	9,4	85		82	84
76.22	550	8,6	83		84	83
76.23	133	8,1	84		83	
76.24	303	8,2	84	84	80	
76.25	27	8,0	53	59		
76.26	69	7,8	34	37		
76.27	9	8,3	49	41		
76.28	4	7,9	72	56		
76.29	432	8,2	79		78	
76.30	41	8,6	78	68		
76.31	104	8,8	59	57	47	
Total	13 669					

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STORABILITY OF *MAESOPSIS EMINII* SEED IN DIFFERENT CONDITIONS

H.P. Msanga

Silviculture Research Station
P.O. Box 95, Lushoto, TANZANIA

ABSTRACT

This paper outlines an experiment to determine appropriate storage conditions which will retain maximum viability of *Maesopsis eminii* seeds. After four months storage, the highest viability (80.5%) was maintained by the seeds which were kept in dehumidified storage at 30 percent relative humidity and at ambient air temperature (18-22°C).

RÉSUMÉ

L'auteur décrit une expérience visant à déterminer les conditions de stockage qui permettent de conserver une viabilité maximale aux graines de *Maesopsis eminii*. Après quatre mois de stockage, les graines conservées dans un entrepôt déshumidifié à la température ambiante (18 à 22°C) ont manifesté la plus forte viabilité (80.5%).

INTRODUCTION

This experiment was a preliminary one to investigate appropriate storage conditions which will retain the maximum viability of *Maesopsis eminii* Engl. seeds. Difficulties have been experienced in determining suitable storage conditions. Storage of the seeds seems possible for some months at least when dry (Magini and Tulstrup 1955). Watkins (1960) states that the longevity of the seeds is five months and Shehaghilo (1979) reported that the seeds lose viability within the first six months when stored in sealed containers at room temperature. It is not known how these seeds retain or lose viability at different storage conditions.

Viable seeds of this species should be available in store because if sown seeds fail due to disease, animal damage or unfavourable weather, it will be possible to reseed. The seeds are also stored to make them available for research and other purposes when they are needed.

MATERIALS AND METHODS

Seeds

Fresh and ripe fruits of *M. eminii* were collected from the ground in Lushoto Arboretum. The fruit pulp around the seed was removed by using a knife. Any pulp which remained on the seed was macerated by vigorous stirring and rinsing in fresh water.

To prevent moulding, without applying a preservative, the seeds were dried by spreading them in shallow layers on wire mesh trays in the shade for seven days. There are about 550 - 600 seeds (air-dried) per kilogramme. The moisture content of the seeds was 16 percent as determined by the air - oven 105°C method for 16 hours (I.S.T.A. 1966).

Design of the experiment

The experiment was a 'Randomized-Block Design' with four blocks (replicates). Each replicate had a set of five different storage conditions (treatments). Number of seeds used per plot, treatment, replicate and the total for one test was 50, 200, 250, and 1000 respectively. Seeds in each treatment (except T_5) were packed in perforated polythene material (0.0375 millimetres thick) and stored for two and four months. The polythene material allowed some water vapour and air exchange.

The treatments were:

T_1 - Control - Seeds stored in an open store at ambient air temperature (18-22°C) and 60 percent relative humidity.

T_2 - Seeds stored in a dehumidified store at ambient air temperature (18-22°C) and 30 percent relative humidity.

T_3 - Seeds stored in a refrigerator at a constant temperature of 3 ± 1°C.

T_4 - Seeds stored in a refrigerator at a constant temperature of -3 ± 1°C.

T_5 - Seeds kept in litter on the forest floor in their natural environment.

Viability test

Germination was used to test the viability of the seeds because the information is needed for estimating the germinability for nursery sowing.

The first viability test was carried out to determine the germinability of freshly collected seeds. Subsequent tests were carried out at an interval of every two months. Seeds from each treatment were soaked in tap water for two days and then simultaneously sown in a sifted sand seed-bed in the nursery.

Assessment

The seed-bed was checked every day to note the commencement of germination. A seed was considered 'germinated' when the hypocotyl protruded on the surface of the seed-bed. All treatments were assessed at the same time. All germinants were counted on the commencement of 'germination' and at every seventh day thereafter. 'Germinated' seeds were removed at every count to prevent their being counted twice.

At the termination of each test, a cut test of ungerminated seeds was done so that germinative capacity could be expressed as a percentage of filled seeds. A seed was counted as filled if the embryo was visible (even though rotted).

RESULTS

The germinative capacity of fresh seeds was 89.0 percent. The germination data after two and four months storage are summarized in Table 1.

Seeds which were stored for two months started to germinate in T_2 in the fifth week after sowing. By the eighth week germination had already started in all treatments, except in T_4 which was nil even at the termination of the test after twelve weeks. Maximum germination was reached at nine and ten weeks after sowing: 83.7%, 32.0%, 28.3% and 3.8% for T_2, T_5, T_3 and T_1 respectively.

The germination in seeds which were stored for four months was nil in treatments T_1 and T_4. Maximum germination occurred in the nineth week after sowing: 80.5%, 21.0% and 2.5% for treatments T_2, T_5 and T_3 respectively.

After two months storage, significant differences by the Least Significant Difference (L.S.D.) method were found between the following storage conditions: $T_4 - T_5$, $T_4 - T_2$, $T_1 - T_2$ and $T_5 - T_2$. There were no significant differences between the other storage conditions.

At the fourth month after commencement of storage, there were significant differences between all storage conditions, except between $T_4 - T_1$, $T_1 - T_3$ and $T_3 - T_5$.

DISCUSSION

The results show that *Maesopsis eminii* seeds retain a higher viability when stored in a dehumidified store at ambient air temperature (18-22°C) and 30 percent relative humidity than at other storage conditions described in this study. Seeds which were kept in litter on the forest floor in their natural environment

Table 1. Germination percent of *Maesopsis eminii* seeds after storage for two and four months at different conditions

Storage period	conditions	Germination percent by weeks after sowing								
		4	5	6	7	8	9	10	11	12
2 months	T_1 : (18-22°C) and 60% RH	0.0	0.0	0.0	0.0	1.5	3.1	3.8	3.8	3.8
	T_2 : (18-22°C) and 30% RH	0.0	15.4	59.0	74.4	74.4	82.1	83.7	83.7	83.7
	T_3 : (3 ±1°C) Refrig.	0.0	0.0	0.0	1.6	28.3	28.3	28.3	28.3	28.3
	T_4 : (-3 ±1°C) Refrig.			Nil						
	T_5 : Forest floor	0.0	0.0	11.8	17.6	17.6	17.6	32.0	32.0	32.0
4 months	T_1 : (18-22°C) and 60% RH			Nil						
	T_2 : (18-22°C) and 30% RH	0.0	10.0	23.3	23.3	53.3	80.5	80.5	80.5	80.5
	T_3 : (3 ±1°C) Refrig.	0.0	0.0	0.0	1.8	1.8	2.5	2.5	2.5	2.5
	T_4 : (-3 ±1°C) Refrig.			Nil						
	T_5 : Forest floor	0.0	0.0	0.0	0.0	0.0	21.0	21.0	21.0	21.0

retained a higher viability than those stored at ambient air temperature (18-22°C) at 60 percent relative humidity or in a refrigerator at constant temperatures of -3 ± 1°C and 3 ± 1°C.

The moisture attained by seeds in storage depends largely on the relative humidity of the air surrounding them (Barner 1975). Thus, the seeds stored at 30 percent relative humidity and ambient air temperature (18-22°C) must have attained a characteristic equilibrium moisture content less than that of the seeds stored at 60 percent relative humidity at the same temperature. By lowering the moisture content the metabolic activity is considerably reduced, resulting in a reduction of respiration and consumption of reserve nutrients - a vital factor in the maintenance of viability (Barner 1975).

The loss of viability in refrigerated seeds at a constant temperature of -3 ± 1°C is an indication that the moisture content (16 percent) of the seeds was high enough to allow freezing injury. There is a critical point of temperature and moisture content at which injury from freezing occurs in seeds (Barner 1975).

The drop in viability in seeds which were kept in litter on the forest floor in their natural environment was mainly due to fungal attack.

CONCLUSION

Pending the outcome of other storage experiments, *Maesopsis eminii* seeds should temporarily be stored in a dehumidified store at ambient air temperature (18-22°C) and 30 percent relative humidity. Seeds should not be stored in a refrigerator with a constant temperature of -3 ± 1°C.

Further research is required to ascertain the storage requirements for *Maesopsis eminii* seed, particularly on the interaction between different packaging materials, storage temperature regimes and moisture content of the seed.

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GERMINATION OF *PAULOWNIA* SEEDS AFTER STRATIFICATION, DRY STORAGE, AND PRETREATMENT WITH GIBBERELLIC ACID

S.B. Carpenter, T.R. Cunningham, and N.D. Smith

Department of Forestry, University of Kentucky, U.S.A.

ABSTRACT

Paulownia tomentosa seeds stored by stratification or by dry storage conditions at 4°C had high germination after 3 years; but speed of germination declined rapidly after 4 months. Stratification is recommended over dry storage. Stratification, gibberellic acid (GA_3), and cold dry storage treatments reduced the light requirement of *Paulownia* seeds for germination. Seeds stratified at 4°C for 2 months germinated rapidly in both light and darkness. The GA_3 treatment ranked second in the promotion of germination in both light and darkness, but was most effective in light. After dry storage treatment germination was lower and delayed compared to the other treatments.

RÉSUMÉ

Les graines de *Paulownia tomentosa* conservées par stratification ou entreposage à sec à 4°C ont eu une germination élevée après 3 ans, mais leur vitesse de germination a baissé rapidement au bout de 4 mois. La stratification est préférable à l'entreposage à sec. Les traitements par stratification, par l'acide gibbérellique (GA_3) et par entreposage à sec au froid ont réduit les besoins en lumière des graines de *Paulownia* pour la germination. Les graines stratifiées à 4°C pendant 2 mois ont germé rapidement à la lumière et à l'obscurité; viennent ensuite les graines traitées au GA_3 pour ce qui est de la vitesse de germination tant à la lumière qu'à l'obscurité, mais plus spécialement à la lumière. Dans le cas de l'entreposage à sec, le taux de germination a été plus faible, et le retard de la germination plus grand que dans le cas des autres traitements.

INTRODUCTION

Paulownia, *Paulownia tomentosa*, (Thunb.) Siev. & Zucc. a native tree of China, seems well suited for use in the reclamation of surface-mined land, and it may have some potential for utilization as well (Carpenter 1977). Paulownia wood is highly valued in Japanese markets. It is actively sought by Asian buyers in Kentucky where it regenerates naturally and grows well. Paulownia has a rapid growth rate and seems to be drought and stress resistant on surface-mined sites. Paulownia is a prolific seeder, producing approximately 6,000 seeds per gram and up to 20 million seeds in a year. For optimal germination under controlled conditions, 50 to 100 hours of continuous light were needed to activate the phytochrome of Paulownia seed. A series of brief illuminations alternating with several hours of darkness will also initiate germination (Borthwick *et al.* 1964).

Previous studies have shown that the pre-germination temperature influences the effective length of the light period necessary for the germination of some tree species. Low temperature reduced the light requirement of *Tsuga canadensis* (L.) Z. (Stearns and Olson 1958). Pre-chilling, seedcoat removal, or the use of an oxygen-enriched atmosphere can be used in lieu of light when germinating *Betula pubescens* Z. seed (Black and Wareing 1955). The objective of this study was to examine the effects of stratification, low temperature dry storage and a pretreatment of gibberellic acid (GA_3) on the germination of Paulownia seed.

MATERIALS AND METHODS

Seeds were collected in the fall of 1974 from a single tree growing near the Wolf Creek Dam on Lake Cumberland in south-central Kentucky. Separate lots of seed were treated as follows:

1 - untreated, fresh seeds
2 - fresh seeds treated with GA_3
3 - stratified seed in a 1:1 mixture of peat moss and sand
4 - seed stored dry in 285-ml sealed glass containers.

Germination tests with fresh seeds began within 2 weeks of collection. The gibberellic acid treatment was applied to fresh seeds by soaking them for a one-hour period in a 100 ppm solution. Stratified seed and dry seed were stored in a cold room that was maintained at 4°C. During the following 36-month period, test samples were removed at intervals to determine the effect of storage method and length of storage on the percent germination and germination value.

Fresh seed, both untreated and treated with GA_3, was germinated with continuous light and in darkness in glass petri dishes on Whatman No. 1 filter paper moistened with distilled water. Stratified and dry stored seed was germinated under continuous light and in darkness after a storage period of 2 months. All subsequent germination tests were under continuous light only. A single treatment test consisted of 10 petri dishes containing 25 seeds each. Temperature during the germination period was maintained at 20°C and the continuous light treatment consisted of a cool white fluorescent light source of 1.5×10^4 lux.

Germination was recorded daily, and was defined by the presence of a normal radicle developed to a length at least twice the width of a single seed. Most tests were continued for a 30-day period and, when completed, the treatments and dates were compared by computing percentage germination and germination value (Czabator 1962).

RESULTS

Figure 1 summarizes the effect of treatments on the cumulative germination of Paulownia seeds grown under continuous illumination. All 3 treatments enhanced germination. Stratified seeds and seeds treated with GA_3 began germinating sooner and germination was completed earlier than with dry stored and untreated seed. Stratified seed reached a maximum germination of 98 percent after 20 days in continuous light, while GA_3 treated seed began germination sooner than stratified seeds, but reached a maximum of only 92 percent. Dry stored seed began germinating after 10 days and increased steadily until completion of the test. Untreated seed responded more slowly, but germination rate accelerated after 20 days.

Germination was lower in the dark for all treatments except stratified seed (Figure 2). Stratified seed reached a maximum germination of 98 percent in only 10 days in the dark. Seeds treated with GA_3 achieved only 48 percent germination after 30 days when tested in darkness; however, GA_3 treated seed germinated earlier than dry stored and untreated seed. Germination of dry-stored seed was delayed and significant germination did not occur until after 20 days. Untreated seed began to germinate after 20 days and reached only 11 percent at the end of the test period.

Germination tests run under continuous light and subjected to stratification and dry storage treatments were compared during a 36-month period. Table 1 summarizes the results for the tests and indicates that Paulownia seed can survive relatively long periods of stratification and dry storage at 4°C with little loss of viability.

While results indicate that percent germination remains high, germination values indicated that speed of germination declined with storage. For stratified seeds, a maximum germination value of 215.50 was reached in only 4 months and declined to 33.12 at the 27-month sampling date. The results for seeds stored dry show a similar trend, but germination values were lower and more erratic. The increase in germination value between the second and fourth month sampling dates may reflect the effect of low temperature storage on the light requirement of Paulownia seeds. This supports the early results indicating that stratified seeds germinate more quickly than unstratified ones.

DISCUSSION

Our results indicate that Paulownia seed should be sown in the field in the fall or they should be stratified prior to sowing in the spring if rapid maximum germination is to be obtained. It also appears that Paulownia seeds can be stored for a relatively long period of time at 4°C without significantly reducing total germination. Although germination percentages over-time remain fairly constant for stored seed, germination values are inversely influenced by time of storage. Stratification is recommended, but storage periods should be limited to 18 months to avoid a decline in germination energy.

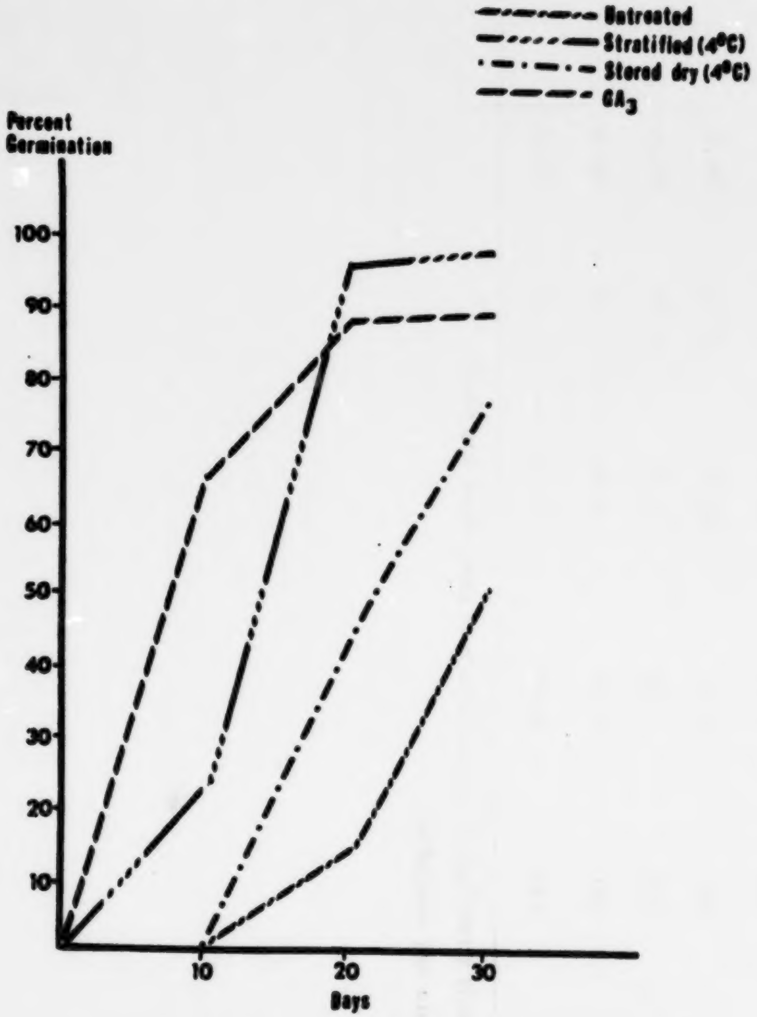

Figure 1. Effect of stratification, GA_3, and cold dry storage treatments on the cumulative germination of Paulownia seeds in the light.

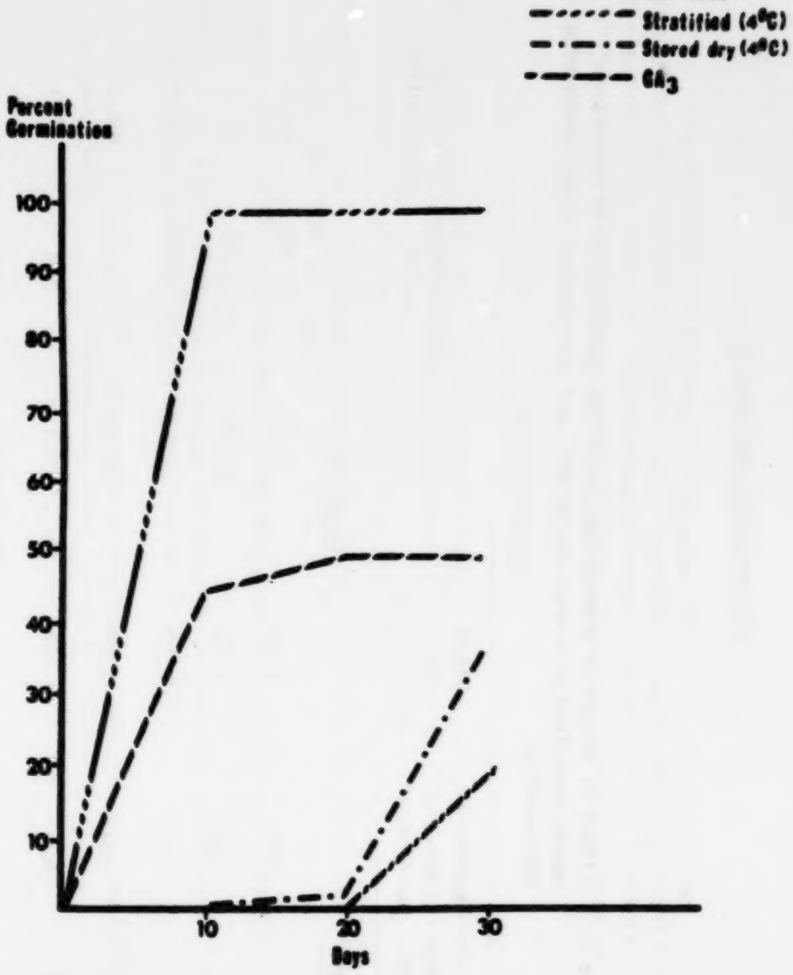

Figure 2. Effect of stratification, GA_3, and cold dry storage treatments on the cumulative germination of Paulownia seeds in the dark.

Table 1. Effect of length of storage period on the germination of Paulownia seeds stratified or stored dry at 4°C and germinated under continuous illumination.

Storage period	Number of seeds germinated	Mean daily germination	Peak value	Germination value	Percentage germination
Months			Stratified[1]		
2	242	5.69	9.28	52.80	96.8
4	246	9.84	21.90	215.50	98.4
18	235	7.23	10.13	73.25	94.0
27	244	4.87	6.80	33.12	97.6
36[2]	NA	NA	NA	NA	NA
			Dry Storage		
2	170	2.58	2.78	7.17	85.0
4	237	5.93	10.05	59.55	94.8
18	214	4.28	6.63	28.38	85.6
27	241	3.57	4.84	17.28	96.4
36	234	4.07	5.71	23.24	93.6

[1] Seeds stratified in a 1:1 mixture of peat moss and sand.

[2] Data not available.

ACKNOWLEDGEMENTS

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STORAGE OF PRECHILLED *ABIES* SEEDS

D.G.W. Edwards
Canadian Forestry Service
Pacific Forest Research Centre
Victoria, B.C.

ABSTRACT

Two seedlots of grand fir and one each of subalpine and amabilis firs were prechilled, then dried to four moisture content levels and stored at 2°C. Seeds not dried had a moisture content of 45% or higher and could be stored only for 3 months before germination began. Losses in viability occurred when they were stored for more than 6-9 months. Seeds air-dried to a 35% moisture content were successfully stored for 6 months, but germination during storage occurred when they were stored for 9 or 12 months. Seeds air-dried to 25% were stored for 12 months without any significant reduction in germination. At both 25% and 35%, the beneficial stimulatory effect of prechilling on germination rate was retained. Oven-drying to 15% moisture prolonged storage life, but the seeds did not retain the prechilling effect. The increases in germination, particularly germination rate, when prechilled seeds were air-dried and stored for 3 months or longer, were sufficiently large to suggest that such treatments might be used for preparing seeds of these species for nursery sowings.

RÉSUMÉ

Deux lots de graines du sapin grandissime, un lot du sapin subalpin et un du sapin gracieux ont été prérefroidies, puis séchées jusqu'à une teneur en humidité de 45, 35, 25 ou 15% et conservées à 2°C. Non séchées, les graines avaient une teneur en humidité d'au moins 45% et ne pouvaient être conservées que 3 mois avant le début de la germination. Des pertes de viabilité se produisaient lorsqu'elles étaient emmagasinées plus de 6 à 9 mois. Les graines séchées à l'air jusqu'à une teneur en humidité de 35% ont pu être conservées 6 mois, mais il y a eu germination lorsqu'elles étaient entreposées 9 ou 12 mois. Les graines séchées à l'air jusqu'à 25% d'humidité se sont conservées 12 mois sans aucune réduction importante de la germination. À 25 et 35% d'humidité, l'effet stimulant du prérefroidissement sur le taux de germination s'est conservé. Le séchage à l'étuve jusqu'à 15% d'humidité a prolongé la durée de conservation, mais les graines n'ont pas gardé l'effet du prérefroidissement. Les augmentations de germination, surtout en ce qui concerne le taux, dans le cas des graines prérefroidies, séchées à l'air et entreposées au moins 3 mois, ont été suffisamment importantes pour qu'on puisse proposer l'utilisation de tels traitements pour la préparation des graines de ces essences en vue de l'ensemencement en pépinières.

INTRODUCTION

One of the most commonly applied pre-sowing treatments for breaking tree seed dormancy is prechilling (also known as stratification), which consists of soaking the seeds in water for several hours, then refrigerating them. Depending upon the species, the refrigeration period may last for a few weeks or several months and timing its completion to coincide with the proposed sowing date can create problems in the nursery. Delayed sowing prolongs the prechill treatment period and some seeds may germinate in the refrigerator before they can be sown. Circumstances sometimes prevent sowing all the seeds that have been prechilled, necessitating a decision whether to discard the surplus or to dry and return them to storage.

A few reports (Vanesse 1967; Hedderwick 1968; Barnett 1972) have indicated that prechilled seeds may be dried and stored at low temperature without losing viability. Where moisture content of the prechilled seeds was reduced to 10% or less, dormancy was reinduced; i.e., the effect of the prechilling treatment was lost. McLemore and Barnett (1968) observed that dormancy in loblolly pine *(Pinus taeda* L.) was greatest when seeds were stored at 10-18% moisture. Dormancy was less at both higher and lower moisture contents, being least when seeds were stored above 20% moisture.

More recently, Danielson and Tanaka (1978) reported that prechilled ponderosa pine *(Pinus ponderosa* Laws.) seeds that had been air-dried to approximately 26% moisture content were successfully stored at 2°C for 9 months without any reduction in viability and without losing the prechill effect. Similarly prechilled seeds of Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco) air-dried to 37% moisture content could be stored for 3 months, but germination occurred during longer periods of storage. Without the air-drying treatment, prechilled seeds of both species had begun to germinate by the third month of cold storage because of their higher moisture contents (33% in ponderosa pine, 50% in Douglas-fir). The practical implications of these findings are twofold. One, seeds might be prechilled (and air-dried) relatively independently of sowing date. For example, Douglas-fir seeds could begin a 1-month prechill treatment any time up to 4 months before sowing; for ponderosa pine, the start of the prechill could be advanced considerably further. Second, the sowing date would be made more flexible since the nurseryman would have a 3-month period in which to sow prechilled Douglas-fir seeds.

Similar advantages might occur if seeds of other species could be handled in the same manner. The research reported here examined the effect of various drying treatments on the storability of prechilled seeds of amabilis (Pacific silver) fir *(Abies amabilis* (Dougl.) Forbes), grand fir (A. *grandis* (Dougl.) Lindl.) and subalpine fir *(A. lasiocarpa* (Hook.) Nutt.).

MATERIALS AND METHODS

Four seedlots of *Abies* spp. were provided by the British Columbia Ministry of Forests. Included were two lots of grand fir collected in seed zones (Dobbs *et al.* 1976) 1020 and 1050 at elevations of 106 m and 254 m, respectively, one lot of amabilis fir seeds from seed zone 1090 at 101 m and subalpine fir seeds from seed zone 1060 at 1110 m.

Prior to experimental work, seedlots were examined using x-ray techniques and all empty and insect-attacked seeds were removed. Seeds to be prechilled were soaked in water for 48 hours, drained, placed in plastic bags and refrigerated at 2°C for 28 days. Following this treatment, seeds were dried to four moisture levels, placed in dry plastic bags and returned to storage at 2°C for periods up to 12 months. The highest moisture level, which consisted of seeds receiving no drying treatment after prechilling, lay between 40%-50% in all seedlots; it is designated as 45% for convenience. Some seeds were air-dried at room temperature for 1-4 hours until the moisture content had decreased to 35%. Others were air-dried for 6-12 hours to 25%, while the lowest moisture level, 15%, was reached after oven-drying at 30°C. Moisture contents were calculated on a fresh-weight basis after drying for 24 hours at 105°C. The three lower moisture levels were achieved by monitoring the change in fresh weight of the samples during the drying treatment. Initially, 10 samples of 50 seeds each from each lot were dried for 24 hours at 105°C, and the mean dry weight was calculated. This mean dry weight was used with each designated moisture content (35%, 25%, 15%) to calculate the fresh weight to which similar sized samples of prechilled seeds had to be dried. Subsequent checks showed that the actual moisture contents reached by this method varied ± 2.5%.

Germination tests were conducted on 4 replications of 50 seeds each, at a daily alternating temperature of 30°C for 8 hours and 20°C for 16 hours, in clear, covered plastic dishes containing one layer of Kimpak (a highly absorbent, multi-layered cellulose wadding) topped by three layers of white filter paper (Whatman no. 1) and 42 ml of distilled water. Light, from cool-white fluorescent tubes, was provided during the high temperature period. Germinants were counted daily during the peak germination period, then every second day, up to 28 days and germinants were evaluated according to the International Rules for Seed Testing (Anon. 1976). Germination percentages were transformed to arcsin and subjected to analysis of variance. Means were compared using the Student-Newman-Keuls' test (Steel and Torrie 1960).

RESULTS AND DISCUSSION

Prechilling, like most other presowing treatments, is designed to stimulate germination rate, which it does effectively for most *Abies* seedlots; in some cases, total germination may also be increased (Franklin 1974). The 28-day prechill applied here (without drying) increased germination rate, as measured by germination percentages at 14 days, by a factor of between 2 and 3.5 in seeds stored for 0 weeks. However, final germination percentages at 28 days were reduced compared to unprechilled seeds (Table 1).

Storage of prechilled but non-dried seeds amounted to a continuation of the initial prechill treatment, so seeds stored for 4 weeks at 45% moisture had been prechilled, in effect, for 2 months. Considerable variability occurred in the germination of such seeds, in terms of both variation among replications at any one storage period as well as between storage periods (Tables 2-4). Variable germination after prechilling for several months has been observed in other studies and is probably related to uncontrolled, higher-than-optimum moisture contents (Edwards, unpublished data). In both *A. grandis* lots stored at 45% moisture, germination began before the 6-, 9- and 12-month storage periods were complete. Germination during storage (shown in parentheses in Tables 2-4), which accounted for almost all the seedlings produced at 9- and 12-month storage, was included in final germination counts. The reduction in final germination in non-dried seeds after these storage periods reflects a loss in seed viability.

Table 1. **Effect of 28-day prechilling, without subsequent drying and storage, on germination of three *Abies* seedlots**

Seedlot	Seed Treatment	Germination after 14 days	Germination after 28 days
Ag 2903	unprechilled	4.0	61.0
	prechilled	15.1	48.0
Ag 2899	unprechilled	13.0	89.5
	prechilled	49.9	79.5
Al 2900	unprechilled	4.1	20.1
	prechilled	10.0	15.5

In all four seedlots, seeds that had been air-dried to either 35% or 25% moisture content prior to storage germinated significantly better than those not dried (45%) or those oven-dried to 15% (Tables 2-5). Tests on *A. amabilis* were limited by the number of filled seeds available to four storage periods and two moisture levels. Air-drying to 35% prior to storage reduced emergence during storage, but did not eliminate it. However, deterioration in seed viability at 9 and 12 months storage was less than that for non-dried seeds. Air-drying to 25% prevented emergence and enabled the seeds to be stored successfully for 12 months, although germination in most cases was significantly lower than in seeds stored at 35% moisture. Oven-dried seeds (15% moisture) stored about as well as seeds not dried (45%), but not as well as air-dried seeds. The reduction in final germination at low moisture content was believed to be due largely to the reinduction of dormancy, rather than deterioration since most of the ungerminated seeds remained viable, as determined by tetrazolium tests. Except for *A. amabilis* (Table 5), for which long storage was not tested, seeds were stored for 6 months without a significant reduction in average germination, irrespective of moisture level. In *A. lasiocarpa*, storage beyond 4 weeks promoted significantly higher average germination, irrespective of moisture level. This was a different response from that in *A. amabilis* and *A. grandis* and may be related to the fact that *A. lasiocarpa* is an alpine species in which a deeper seed dormancy may be encountered.

The stimulatory effect of prechilling on germination rate was retained by all species when they were air-dried to 25% and 35% moisture and stored. Final germination percentages were also increased by these treatments, with maximum values for both rate and final germination occurring at 35% with 3-month storage in the two *A. grandis* lots (Tables 2 and 3) and 6-month storage in *A. lasiocarpa* seeds (Table 4). Germination was so rapid in seeds air-dried to 35% that it was essentially complete (within 0.5% of the final germination percent) at 14 days when seeds were stored for 3 months or longer. These germination rates were approximately 2-7 times greater than in seeds prechilled for 28 days (but not dried) and were 6.5-17 times greater than in unprechilled seeds. In seeds not dried (45%), germination was essentially complete at 14 days only in seeds stored for 6 months or longer, by which time losses in viability had begun. Especially in seedlots Ag 2899 (Table 3) and Al 2900 (Table 4), storage for 9 and 12 months of non-dried seeds reduced germination rates below those in unprechilled seeds (Table 1). Other trials on approximately 30 additional seedlots have confirmed that air-drying prechilled *Abies* seeds to 35% moisture followed by 3-month storage produces better germination than prechilling alone (Edwards and Leadem, unpublished).

Table 2. **Germination (%) of *Abies grandis* seeds (seedlot Ag 2903) at four moisture levels and nine storage periods following prechilling**

	Moisture Content				
Storage period	45%	35%	25%	15%	Germination means[1]
		Germination After 14 Days			
0 wk	15.1	29.6	24.3	24.1	23.3 b*
1 wk	31.5	66.0	53.5	33.0	46.0 a
2 wk	30.3	72.5	42.5	21.0	41.6 a
3 wk	28.5	76.0	49.5	29.0	45.8 a
4 wk	33.5	83.0	47.5	22.0	46.5 a
3 mo	34.5	85.0	47.0	26.5	48.3 a
6 mo	68.0(51.5)[2]	81.0	34.5	11.5	48.8 a
9 mo	54.0(52.0)	81.0(10.5)	27.5	7.5	42.5 a
12 mo	25.0(25.0)	74.5(16.5)	46.8	7.5	38.4 a
Germination means[1]	35.6 b*	72.1 a	41.4 b*	20.2 c*	
		Germination After 28 Days			
0 wk	48.0	62.5	66.0	74.0	62.6 ab
1 wk	61.5	84.3	78.0	68.8	73.1 a
2 wk	60.5	87.0	71.3	63.5	70.6 a
3 wk	52.5	81.5	70.5	69.5	68.5 ab
4 wk	63.8	87.0	73.5	62.5	71.7 a
3 mo	39.8	85.0	70.3	59.3	63.6 ab
6 mo	71.0(51.5)	81.0	59.8	46.5	64.6 ab
9 mo	54.0(52.0)	81.5(10.5)	57.5	35.5	57.1 b
12 mo	25.0(25.0)	75.0(16.5)	66.8	22.5	47.3 c
Germination means[1]	52.9 c*	80.5 a	68.2 b	55.8 c*	

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[1] Means followed by the same letter are not significantly different (P = 0.05). An asterisk indicates P = 0.01.

[2] Figures in brackets indicate germination % during storage (included in total germination).

Table 3. Germination (%) of *Abies grandis* seeds (seedlot Ag 2899) at four moisture levels and nine storage periods following prechilling

	Moisture Content				
Storage period	45%	35%	25%	15%	Germination means[1]
		Germination After 14 Days			
0 wk	49.9	63.5	63.1	45.1	55.4 a
1 wk	40.0	71.0	67.0	38.5	54.1 a
2 wk	40.3	73.8	64.5	41.5	55.0 a
3 wk	32.0	80.0	65.5	45.0	55.6 a
4 wk	45.0	75.0	61.5	39.5	55.3 a
3 mo	49.5	91.5	72.0	43.0	64.0 a
6 mo	61.0(48.0)[2]	82.0(17.0)	53.5	19.0	53.9 a
9 mo	10.5(10.5)	51.5(32.5)	71.0	13.0	36.5 b
12 mo	38.0(38.0)	62.1(29.0)	38.1	10.5	37.2 b
Germination means[1]	40.7 b*	72.3 a	61.8 a	34.2 b*	
		Germination After 28 Days			
0 wk	79.5	85.3	82.5	72.8	80.0 a
1 wk	61.5	76.8	85.5	68.8	73.1 a
2 wk	66.8	79.5	80.0	76.0	75.6 a
3 wk	48.8	82.5	77.3	73.0	70.4 a
4 wk	62.8	77.0	71.5	67.3	69.6 a
3 mo	53.5	92.0	79.8	63.0	72.1 a
6 mo	61.5(48.0)	82.3(17.0)	70.3	52.8	66.7 a
9 mo	10.5(10.5)	51.5(32.5)	82.5	55.0	49.9 b
12 mo	38.5(38.0)	63.3(29.0)	56.5	31.0	47.3 b
Germination means[1]	53.7 b*	76.7 a	76.2 b	62.2 b*	

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[1] Means followed by the same letter are not significantly different (P = 0.05). An asterisk indicates P = 0.01.

[2] Figures in brackets indicate germination % during storage (included in total germination).

Table 4. Germination (%) of *Abies lasiocarpa* seeds (seedlot A1 2900) at four moisture levels and nine storage periods following prechilling

Storage period	Moisture Content 45%	35%	25%	15%	Germination means[1]
		Germination After 14 Days			
0 wk	10.0	17.3	12.3	6.3	11.4 c
1 wk	14.0	18.5	11.5	8.0	13.0 c
2 wk	11.5	25.3	11.0	1.5	12.3 c
3 wk	18.5	22.5	17.5	8.5	16.8 bc
4 wk	21.5	32.0	28.0	2.5	21.0 b
3 mo	13.0	69.0	39.0	6.0	31.8 a
6 mo	22.0	73.5	57.5	3.0	39.0 a
9 mo	4.5	70.5	61.6	1.0	34.4 a
12 mo	0.0	65.5(15.5)[2]	61.0	10.0	34.2 a
Germination means[1]	12.8 c*	43.8 a	33.3 b*	5.2 d	
		Germination After 28 Days			
0 wk	15.5	25.8	23.0	15.0	19.8 c
1 wk	26.8	27.8	23.8	16.5	23.5 bc
2 wk	19.5	33.3	27.5	12.0	23.1 bc
3 wk	25.3	34.3	34.3	22.0	28.9 b
4 wk	31.0	42.3	39.8	10.0	30.8 b
3 mo	24.5	69.5	47.8	19.8	40.2 a
6 mo	31.3	73.5	59.0	8.5	42.9 a
9 mo	5.0	70.5	64.0	11.0	37.6 a
12 mo	0.0	65.5(15.5)	62.0	27.0	38.6 a
Germination means[1]	19.9 c*	49.0 a	42.3 b*	15.7 c*	

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[1] Means followed by the same letter are not significantly different (P = 0.05). An asterisk indicates P = 0.01.

[2] Figures in brackets indicate germination % during storage (included in total germination).

Table 5. **Germination (%) of *Abies amabilis* seeds (seedlot Aa 2717) at two moisture levels and four storage periods following prechilling**

	Moisture Content		
Storage period	45%	35%	Germination means[1]
	Germination After 14 Days		
0 wk	6.7	18.3	12.5 b
2 wk	6.3	43.7	25.0 ab
4 wk	0.0	50.7	25.3 ab
3 mo	12.0	57.3	34.7 a
Germination means[1]	6.3 b*	42.5 a	
	Germination After 28 Days		
0 wk	37.0	49.3	43.2 a
2 wk	27.7	65.7	46.7 a
4 wk	12.0	65.3	38.7 a
3 mo	23.7	61.3	42.5 a
Germination means[1]	25.1 b*	60.4 a	

[1] Means followed by the same letter are not significantly different ($P = 0.05$). An asterisk indicates $P = 0.01$.

This study has indicated that *Abies* seeds can be safely stored for periods up to 12 months after prechilling without significant losses in total germination, or without losing the beneficial stimulation effect, if they are air-dried. Seed moisture content is a critical factor governing the period of storage. To prevent germination in storage, moisture levels must be reduced to 25%; maximum germination occurs at 35% and 3-month storage, but this moisture content is unsuitable for storing seeds beyond 6 months. An intermediate moisture level of 30% might promote high germination and allow longer storage. These results are supported by the findings of McLemore and Barnett (1968) that *Pinus taeda* seeds were least dormant when stored at moisture contents of 20% or greater, but that seeds deteriorated more quickly than at lower moisture contents.

Even if there is no requirement to store prechilled seeds, the stimulation of germination achieved by this technique should be advantageous in the nursery. Additional testing is already underway to (i) clarify the optimum moisture levels for storage and stimulation over a wide range of seedlots, (ii) ascertain if the initial prechill period, when seed moisture contents reach 45% or more, can be shortened, and (iii) determine if the procedure can be adapted for use on other tree species.

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ALTERATION OF ENDOGENOUS GROWTH REGULATORS IN COLD-MOIST STRATIFIED SEEDS OF *GINKGO BILOBA L.*

Kyong-Bin Yim and Kyong-Jae Lee

Department of Forestry, College of Agriculture,
Seoul National University, Suwon, Korea

ABSTRACT

The growth regulators in the embryo, endosperm and seed coat of *Ginkgo biloba* which were treated under cold-moist storage for five months, were studied by paper chromatography, thin layer chromatography and bioassay techniques. The dormancy release was due to the decrease in growth inhibitors during the cold-moist storage. Abscisic acid is considered to be the growth inhibitor, since under UV fluorescence lamp, three spots were clearly appeared, purple, gray and blue colours of Rf 0.62, 0.70 and 0.78 respectively.

RÉSUMÉ

Grâce à la chromatographie sur papier, à la chromatographie sur couche mince et à des techniques de bioanalyse, on a étudié les régulateurs de croissance de l'embryon, de l'endosperme et du tégument de graines de *Ginkgo biloba* qui ont été entreposées en stratification froide et humide pendant cinq mois. La fin de la dormance a été due à la diminution des inhibiteurs de croissance au cours de l'entreposage. On pense que l'inhibiteur est l'acide abscissique, car à l'aide d'une lampe à UV, on a pu observer clairement trois taches, de couleurs pourpre, grise et bleue, dont le Rf était respectivement de 0.62, 0.70 et 0.78.

INTRODUCTION

Ginkgo biloba seedlings are raised and planted widely as street and park trees. Seeds are a valuable cooking material in Korea. Seeds after collection during October to November are stored either in a dry condition in the room or under open ground. When stored under cold-moist condition, more regular and quick germination at nursery in spring is expected. This storage environment results in chemical changes in the seeds needed to break dormancy.

In this study, alteration of endogenous growth substances in cold-moist stratified seeds is analyzed and compared with that of dry seeds as a control.

With regard to the growth inhibitors a number of reports have appeared (Bennet-Clark and Kefford 1953; Bennett and Bonner 1953; Eagles and Wareing 1963; Kim 1966; Phillips and Wareing 1958; Shibaoka and Imaseki 1957; Walker *et al.* 1957). Cornforth *et al.* (1965) separated dormin from *Platanus* leaves and named it Abscisin 11, i.e., Abscisic acid (ABA). Hatano and Miyazaki (1974) studied ABA with *Sciadopitys verticillata* seeds which is native to Japan, and Lipe and Crane (1966), with peach seeds which were stored under cold-moist conditions for six months. The decrease of ABA concentration in seeds during cold storage was reported by many researchers (Martin *et al.* 1969; Sondheimer *et al.* 1968; Webb and Wareing 1972).

MATERIAL AND METHODS

Materials

The seeds were collected on October 17 and dried under shade after removing the fleshy outer seed coat, and then stored under open ground for five months without any frost damage. The seeds used as a control were kept in a dry room condition. Only sound seeds were used.

Methods

(1) Extraction of growth regulators.

Just prior to the experiment, the seeds were kept in a refrigerator for 24 hours at -15°C to -18°C after 5 months storage. Samples of 20 grams of seed, seed coat, embryo and endosperm, were ground and extracted with 100 ml of 80% ethanol for 24 hours. These procedures were repeated 3 times requiring 72 hours, then filtered. The ethanol extracts were concentrated using a vacuum-rotary-evaporator at under 35°C and then dissolved in 2 ml of anhydrous ethanol and kept in a refrigerator for paper chromatography.

(2) Separation of growth regulators by paper chromatography.

Whatman No. 1 paper, 2 x 29 cm in size, was used for one-dimensional ascending chromatography. An extract of 0.1 cc was in the starting point. The running distance was 20 cm at 25°C under dark. The developing solvent was Isopropanol: 28% ammonia: water (40:5:5v/v).

(3) Bioassay of growth regulators.

The technique was followed by the method described in a previous report (Yim 1962). However, the coleoptiles of rice seedlings (Early tong-il, Suweon 213-E417-B-1) were used.

(4) Extraction of growth inhibitors.
This was followed by Cornforth's method (Cornforth *et al.* 1965).

(5) Separation of growth inhibitors by paper chromatography.
This was similar to Method No. 1. However, 5 different developing solvents were used.

(i) Isopropanol: water (80:20v/v)
(ii) Benzene: acetic acid: water (8:3:5v/v)
(iii) Ethanol: water: 28% ammonia (4:5:1v/v)
(iv) n-Butanol: 28% ammonia (500:1v/v)
(v) n-Butanol: acetic acid: water (4:4:1v/v)

Because of the abundance of growth inhibitors within the range of Rf 0.5-0.8 on the paper, this paper section was extracted in 80% methanol. The concentrated extracts were developed by thin layer chromatography. The developing solvent was n-propanol:n-butanol: 28% ammonia: water (6:2:1:2v/v). After drying the plate, it was atomized by ethanol: sulphuric acid (95:5v/v), and then inspected under ultraviolet-fluorescence lamp (2,537Å).

(6) Bioassay of growth inhibitors.
This technique is also similar to the above method. However, wheat coleoptiles were used. There were 5 replications, with 10 coleoptile sections of 3 mm long in each replication under darkness for 20 hours of growth.

RESULTS AND DISCUSSION

I. Experiment I. Alteration of growth regulators.

The alteration of growth regulators influenced by open ground storage by parts of the seeds, embryo, endosperm, and seed coat, and analyzed through the rice seedling test are mentioned below.

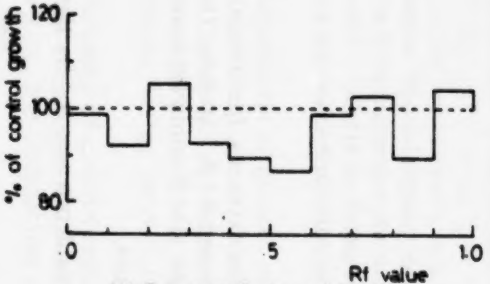
As seen from Fig. 1, the growth inhibitory zone originating from the embryo extracts appeared in the range of Rf 0.3 to 0.7 and growth stimulating zones are not prominent, particulary in the control sample. However, in the stratified embryo, the growth stimulating zone increased in activity between Rf 0.7 to 0.8.

In Fig. 2, irrespective of treated (cold-moist storage) or non-treated (dry storage) conditions, growth inhibitors were always present in the below Rf 0.7 zone. But there was an abrupt change at the point of Rf 0.7. These results imply that the endosperm has a growth inhibiting role, and does not respond to the treatment.

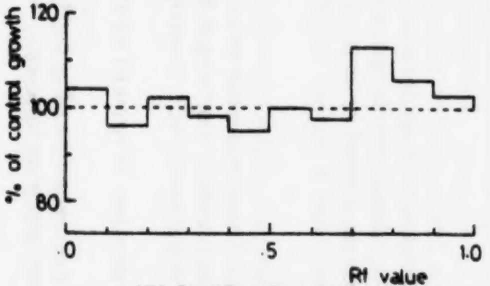
In Fig. 3, with regard to the seed coat extracts, growth inhibitors were present as shown by the bioassay technique between Rf 0.3 to 0.9. The inhibiting effect remained after treatment, but its strength was diminished considerably.

II. Experiment II. Separation of growth inhibitors.

The results of behavior of growth inhibitors assayed by the wheat coleoptile straight-growth test were as follows:

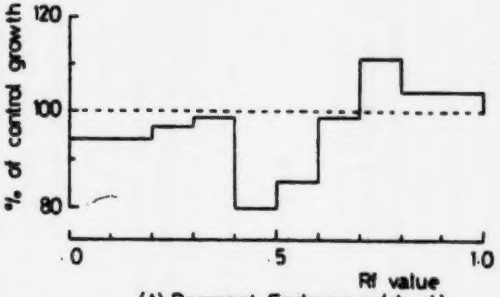


(A) Dormant Embryo (check)

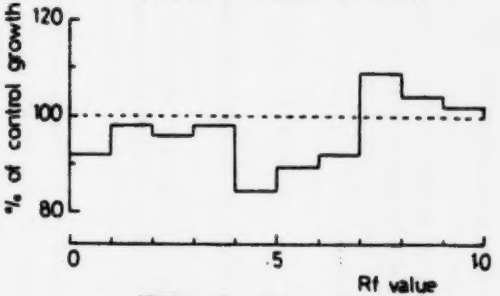


(B) Stratified Embryo(treated)

Fig.1. Histogram of plant growth regulator in dormant(A) and stratified embryo (B) of Ginkgo seeds determined by rice seedling test.

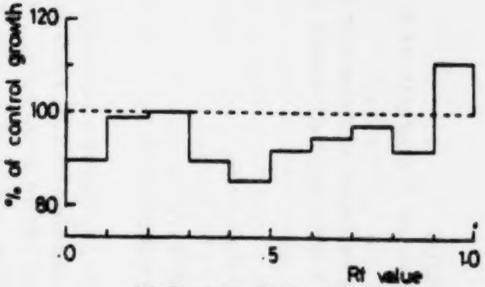


(A) Dormant Endosperm(check)

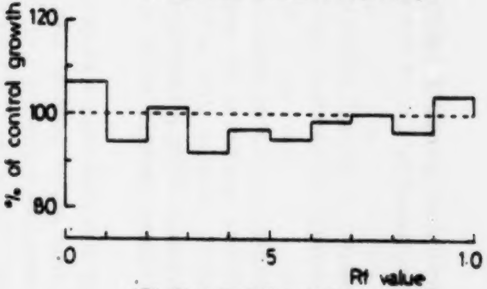


(B) Stratified Endosperm(treated)

Fig.2. Histogram of plant growth regulator in dormant(A) and stratified endosperm(B) of Ginkgo seeds determined by rice seedling test.

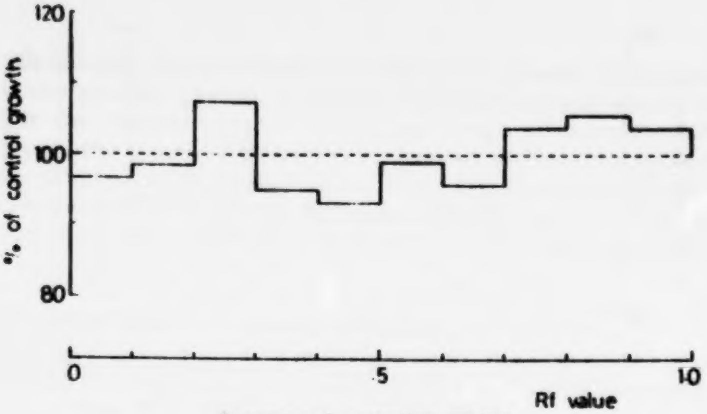


(A) Dormant Seed Coat(check)



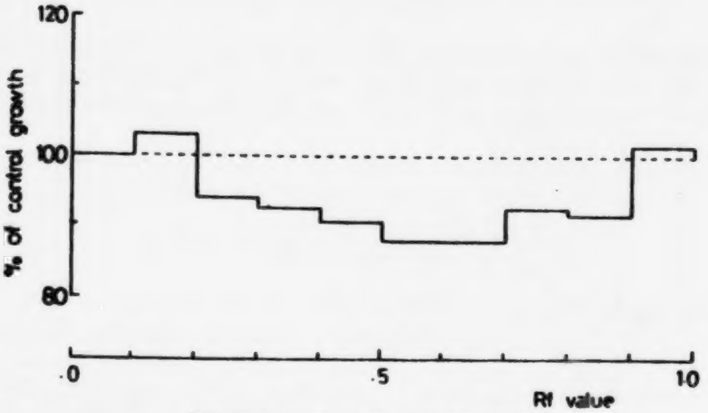
(B) Stratified Seed Coat (treated)

Fig.3. Histogram of plant growth regulator in dormant(A) and stratified seed coat(B) of Ginkgo seeds determined by rice seedling test.



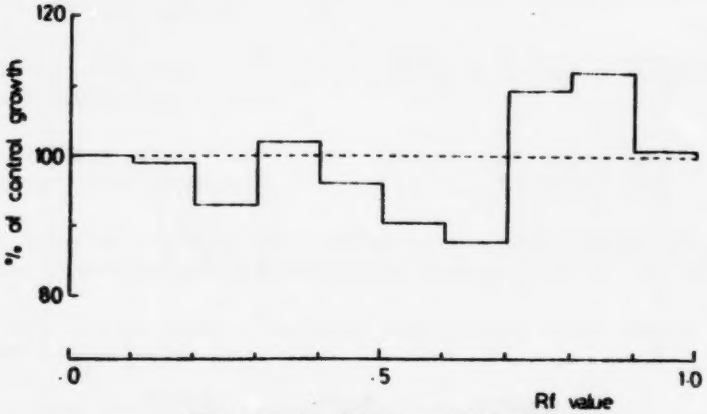
Isopropanol: water (80:20 V/V)

Fig.4. Histogram of growth inhibitor substances of Ginkgo seeds by wheat coleoptile test.



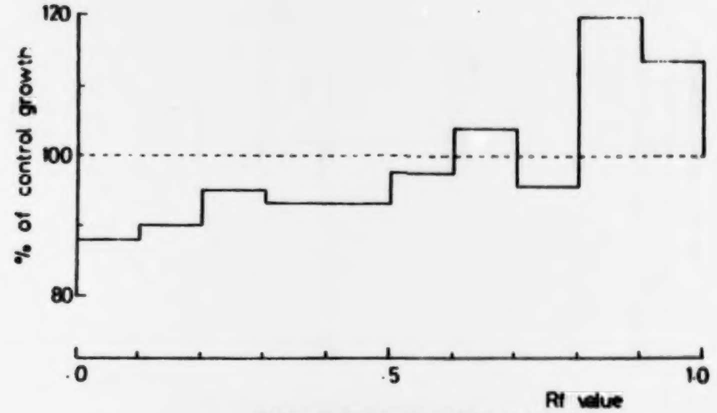
Benzene: acetic acid: water (8:3:5 V/V)

Fig.5. Histogram of growth inhibitor substances of Ginkgo seeds by wheat coleoptile test.



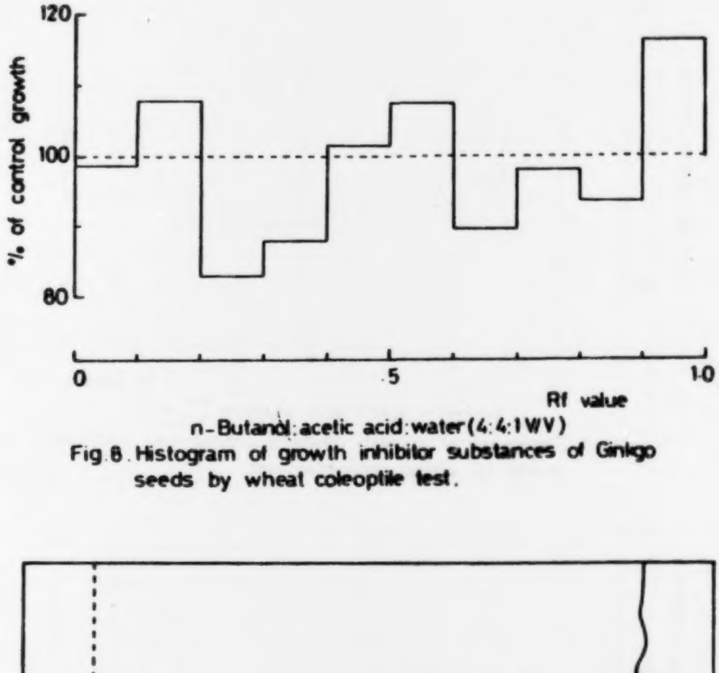
Ethanol: water: 28% ammonia (4:5:1 V/V)

Fig.6. Histogram of growth inhibitor substances of Ginkgo seeds by wheat coleoptile test.



n-Butanol:28%.ammonia(500:1WV)

Fig. 7. Histogram of growth inhibitor substances of Ginkgo seeds by wheat coleoptile test.



n-Butanol:acetic acid:water(4:4:1WV)

Fig. 8. Histogram of growth inhibitor substances of Ginkgo seeds by wheat coleoptile test.

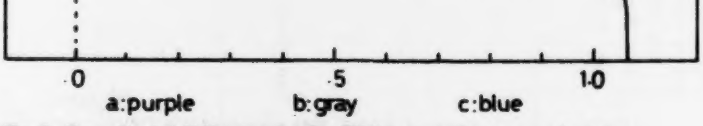


Fig. 9. Growth inhibitor zone in Ginkgo seeds seperated by thin-layer chromatography.

(1) Ultraviolet fluorescence lamp check on growth inhibitors.

Since the greater degree of growth inhibiting effect was in the range of Rf 0.5 to 0.8, in the control material which was prepared from the embryo, endosperm and seed coat, the paper of this section was cut and extracted for thin layer chromatography. Under the UV fluorescence lamp, 3 spots; purple, gray and blue in colour were observed at Rf 0.62, Rf 0.70 and Rf 0.78 (Fig. 9). These spots are thought to be related to the presence of ABA, according to similar results in other reports.

(2) Bioassay results of growth inhibitors.

As seen in Fig's 4,5,6,7 and 8, different developing solvents gave different patterns of inhibiting and stimulating effects by Rf zones when checked by bioassay.

Examining the results, the reason for improved germination is thought to be the result of the decrease of growth inhibitors in the embryo and seed coat through cold-moist treatment. It is thought that the growth stimulators are not important in the breaking of dormancy as their levels increased only in the embryo.

In other reports the decrease of ABA concentration in seeds was also associated with the breaking of seed dormancy (Eagles and Wareing 1963; Kim 1966; Martin *et al.* 1969; Sondheimer *et al.* 1968; Webb and Wareing 1972).

Our research results are, in many aspects, similar to the report of Ramsay and Martin (1970). They extracted ABA from apricot buds.

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LONG-TERM STORAGE OF *ABIES, BETULA, LARIX, PICEA, PINUS* AND *POPULUS* SEEDS

By

B.S.P. Wang
Canadian Forestry Service
Petawawa National Forestry Institute
Chalk River, Ontario
K0J 1J0

ABSTRACT

This study is to test the effectiveness of two cold storage temperatures for long-term storage of seeds of 10 tree species. Results indicated that seeds of balsam fir, white spruce, eastern white pine, yellow birch, aspen and cottonwood can be effectively stored for 10 - 13 years with low seed moisture content in air-tight containers at −18°C; while black spruce, tamarack, jack and red pine seeds can be stored equally well for 11 - 12 years at both 2°C and −18°C.

Plastic containers were found as suitable as glass containers for long-term storage of spruce, pine and small seeded hardwood seeds at −18°C. Long-term stored seeds of black spruce, balsam fir, white pine and yellow birch at −18°C showed deeper dormancy and required longer prechilling pretreatment (3 - 5 weeks) for maximum germination.

RÉSUMÉ

L'objet de l'étude était de contrôler l'efficacité de deux températures d'entreposage frigorifique à long terme des graines de 10 essences. Les résultats indiquent que les graines du sapin baumier, du pin blanc, du bouleau jaune, du peuplier et du peuplier deltoide se conservent bien de 10 à 13 années avec une faible teneur en humidité des graines dans des récipients étanches à l'air à -18°C, tandis que celles de l'épinette noire, du mélèze laricin, du pin gris et du pin rouge se conservent aussi bien pendant 11 à 12 ans à 2° et -18°C.

Les récipients en plastique conviennent aussi bien que ceux en verre pour le stockage à long terme à -18°C des graines d'épinette, de pin et de feuillus à petites graines. Les graines d'épinette noire, de sapin baumier, d'épinette blanche et de bouleau jaune entreposées à -18°C pour de longues périodes ont manifesté une dormance plus profonde et demandé un refroidissement préalable plus long (3 à 5 semaines) pour donner une germination maximale.

INTRODUCTION

Storage of forest tree seed is a necessity because of seed crop periodicity in forest trees. For continuous production of planting stock and container seedlings and for direct seeding programs, some provincial forestry services in Canada such as British Columbia always maintain sufficient quantities of seeds of various important reforestation species for 10 years' supply. These quantities of seeds are usually collected in good crop years and stored to maintain germinability.

The storage life of tree seed is known to be affected by many factors beginning from the time of harvesting throughout the various stages of handling and processing to storage conditions. Many previous studies on storage of orthodox tree seeds suggest good initial seed quality with low moisture content (less than 10%), low storage temperature (5°C to −18°C) and in sealed or air-tight storage containers (Allen and Bientjes 1954, Barton 1961, Harrington 1972, Huss 1967, Rudolf 1952). Allen (1957) investigated seed storage life of 11 western Canadian tree species under two storage temperatures and found all seeds can be stored equally well at both 0°C and −18°C for 5 to 7 years with negligible losses.

To test the effectiveness of the two storage temperatures of the National Tree Seed Centre's storage facilities at the Petawawa National Forestry Institute for various eastern and western Canadian tree seeds, cones and fruits of 41 seedlots and species were collected from 1966 to 1969, carefully handled and processed and stored at 2°C and −18°C. The germinability of the stored seeds were tested periodically to detect any changes.

This paper reports the results of the last test in 1980, an interval of 10 to 13 years.

METHODS AND MATERIAL

Cones of all coniferous species were collected from standing and felled trees, air-dried in a well-ventilated, cool cone shed for two to four months, and carefully processed with moist dewinging and conditioning before storage. Cones of jack pine *(Pinus banksiana* Lamb.), black spruce *(Picea mariana* B.S.P.), red pine *(Pinus resinosa* Ait.) and eastern white pine *(Pinus strobus* L.) were kiln dried at a temperature of 50 - 60°C for 16 hours. All seeds were conditioned at 26°C for 16 hours before bottled in air-tight glass jars. Fruits of yellow birch *(Betula alleghaniensis* Britton), plains cottonwood *(Populus deltoides* var. *occidentalis* Rydb.) and largetooth aspen *(Populus grandidentata* Michx.) were collected from standing or felled trees, air-dried, cleaned and stored. Seeds of several seedlots were stored in plastic jars to test its efficiency. Seeds of most species were stored under two storage temperature conditions: +2°C and −18°C.

Moisture contents and germination tests of stored seeds were determined according to the standard procedures prescribed in the International Rules for Seed Testing (International Seed Seed Testing Association 1976). Initial seed germinability as shown in Tables 1 and 2 are those of earlier tests which had been made most closely in accordance with the prescribed standard procedures in the same year as the seeds were processed with some exceptions.

From each seedlot four replicates of 100 seeds were germinated on top of blotter paper over a layer of Kimpak in plastic germination boxes. Both ends of the Kimpak touch a layer of water at the bottom of the box and serve as a wick. Germination counts were made weekly and seeds germinated with healthy roots and raised hypocotyl were considered as germinated normal seedlings. Seeds germinated only with radicles were considered as low vigour.

RESULTS AND DISCUSSION

Effects of storage temperature on germinability of coniferous and hardwood seeds after prolonged period of 10 - 13 years storage are shown in Tables 1 and 2.

In coniferous seeds balsam fir, white spruce and eastern white pine were stored better at −18°C than at 2°C, whereas tamarack, jack pine and red pine and black spruce maintained their germinability equally well or better at 2°C than at −18°C. For balsam fir seed, the reduction in germinability appears to have occurred after 3 years' storage. The loss of the original germinability was from 74% to 43% at −18°C as compared to that from 74% to 4% at 2°C after 12 years storage. The superiority of sub-freezing temperature −18°C, low moisture content (6 - 9%) and sealed containers for long-term storage of noble fir (*Abies procera* Rehd.) was reported previously by Danielson and Grabe (1973), although Holmes and Buszewicz (1962) stressed the importance of low moisture content and sealed containers for one year storage. However, Allen (1957) investigated the effect of storage temperature of 0°C and −18°C for 11 western tree seed species and found that silver fir (*Abies concolor* (Gord. & Glend.) Lindl.) seed stored better at −18°C while noble fir seed stored equally well at both temperatures. It seems to be possible that balsam fir seed could be stored effectively for 10 years when seed moisture content is reduced to 6 - 9% and stored in air-tight or sealed containers at −18°C.

It was interesting to note that balsam fir seed became more dormant after a prolonged period of storage at −18°C. The 1977-78 tests indicated that balsam fir seeds stored for 10 years at −18°C germinated with 10% normal seedlings, 5% low vigour and 31% fresh ungerminated seeds following 3 week prechilling as compared to 26% normal seedlings, 4% low vigour and only 2% fresh ungerminated seeds of those stored at 2°C (Table 1). When the same seedlot was re-tested in 1980 after 12 years' storage at −18°C, it germinated with 40% normal seedlings, 7% low vigour and only 2% fresh ungerminated seeds following 5 weeks prechilling (Table 1).

Tamarack seeds stored very well at 2°C for 10 years with germinability reduced from 100% to 96%. It is believed that both the high initial seed quality and low seed moisture content (7.4%) were the contributing factors to the maximum retention of the original seed germinability in air-tight containers.

Seeds of black spruce and jack and red pine appeared to store equally well for 12 - 13 years at both 2°C and −18°C, although two lots of black spruce showed lower germination at −18°C than at 2°C in the 1977-80 tests (Table 1). The difference in germination tests between 1978 and 1980 of black spruce seedlots 2 and 3 stored at −18°C was probably due to sampling variation of empty seeds and the difficulty in correctly classifying empty from dead seeds in cutting tests of ungerminated seeds at the end of prescribed test periods (Haddon unpublished report 1979). The 1980 x-radiographs showed that the black spruce seedlots stored at -18°C were cleaned to only 70 to 90% filled seeds while the seeds stored at 2°C were recleaned to 100% filled seeds.

Table 1. Effect of storage temperature on germinability of coniferous seeds.

Species and seedlot		Collection Year	Moisture Content	Initial Germination	Germination % after various periods of storage by temperatures							
					1969-70	1971-72	1973-74	1975-76	1977-78		1980	
			(%)	(%)	2°C	2°C	2°C	2°C	2°C	-18°C	2°C	-18°C
Abies balsamea		1967	11.2	74	73	70	-	-	26	10	4	40
Larix laricina		1967	5.5	100	99	-	-	-	96	-	-	-
Picea glauca	1	1966	7.4	94	-	86	92	-	-	-	85	-
	2	1966	7.3	89	-	90	-	-	-	-	84	-
	3	1967	5.5	75	-	-	74	-	73	78	74	88
Picea mariana	1	1967	6.6	98	-	98	96	-	99	97	-	97
	2	1967	6.2	99	-	-	-	-	97	66	92	79
	3	1967	5.6	100	-	-	-	-	100	89	93	95
Pinus banksiana		1968	8.3	95	-	-	-	94	96	96	94	99
Pinus resinosa	1	1968	6.3	87	90	81	83	-	84	61	84	80
	2	1968	12.6	66	65	-	60	-	24	-	3	-
Pinus strobus	1	1966	8.6	76	79	79	-	81	78	77	72	84
	2	1966	7.3	93	-	95	-	97	-	-	86	-
	3	1966	6.8	98	-	97	-	-	98	-	84	-
	4	1967	6.5	-	-	-	-	98	81	98	81	83
	5	1966	6.0	98	-	98	-	98	-	-	78	-
	6	1966	5.6	81	-	81	-	-	37	46	44	60
	7	1966	4.8	71	-	89	-	83	63	57	42	35

Results of previous storage research has shown that white and black spruce and jack pine seeds can be safely stored for 21 - 22 years (Wang 1974, Wang and Haddon 1976) and red pine 42 years (Eliason and Heit 1973). The rapid loss of germinability of red pine seedlot 2 after 5 years' storage was obviously due to poor initial seed quality and high moisture content (12.6%) (Table 1). These findings clearly suggest that seeds of this species group can be effectively stored for 20 - 30 years when they are stored with a moisture content of 5 to 8% in air-tight or sealed containers at near freezing temperature.

Seeds of eastern white pine appeared to store better at −18°C than at 2°C for 12 - 13 years. It is interesting to note from Table 1 that the greatest losses of germinability occurred in seedlots 5 to 7 which had lower moisture contents ranging from 4.8% to 5.6%. Maybe eastern white pine seed has a higher lower limit of critical moisture content for long-term storage.

Small seeded hardwood seeds of yellow birch, aspen, and cottonwood can tolerate drying equally well as coniferous seeds thus can be stored effectively for long periods at sub-freezing temperature. From Table 2, it is obvious that yellow birch seeds stored best at −18°C with reduction in germinability from 10% to 42% among the 5 seedlots as compared to 29% - 83% from that at 2°C after 12 years of storage. The heaviest loss of germinability of seedlots stored at both 2°C and −18°C were those with high moisture contents, 13.4 - 15%. The difference in germinability of 1978 and 1980 tests was due to the prechilling treatment which was applied to seeds in 1980 tests. It is evident that seeds stored for 12 years at −18°C become more dormant and required 3 week prechilling for maximum germination. According to Clausen's study in Wisconsin, U.S.A. (1975), yellow birch seed can be stored at 2 to 5°C in closed containers for 8 years. Although the results of his first collection showed an increase in germinability of 20% after 4 years' storage and declined 34% and 50% after 8 and 12 years' storage respectively. However, it is not practical to store yellow birch seed for prolonged periods as they are produced frequently and efficiently as pointed out by Clausen (1975).

Populus seeds can only be stored at sub-freezing temperature to retain their germinability for longer than 1 year (Wang and Haddon 1976). Several lots of both *Populus deltoides* var. *occidentalis* and *Populus grandidentata* stored excellently at −18°C after 10 - 11 years. Bensen and Harder (1972) found hybrid aspen seeds stored in sealed coin envelopes over calcium chloride for 4 years at −24°C with little loss of germination, although some lots suffered heavy losses after 4 years storage at 4.4°C. In most cases the loss of seed germinability of *Populus* seeds in this study were in the form of abnormal germinants. The 1977 test showed 9 - 56% of the seeds germinated abnormally. The loss of germinability of *Populus* seeds in storage did not appear to be associated with high seed moisture content (10 - 13.5%). Nearly 50% of the seedlots maintained their original germinability after 4 years' storage.

Plastic containers were found as suitable as glass containers for long-term storage of spruce, pine and yellow birch seeds at −18°C.

Table 2. Effect of storage temperature on germinability of broad-leaved seeds.

Species and seedlot		Collection Year	Moisture Content	Initial Germination	Germination % after various periods of storage by temperatures 1969-70	1971-72	1973-74	1977-78		1980	
			(%)	(%)	2°C	-18°C	2°C	2°C	-18°C	2°C	-18°C
Betula alleghaniensis	1	1967	15.0	85	84	-	8	2	48	1	54
	2	1967	14.3	60	62	-	15	1	22	0	23
	3	1967	13.4	77	76	-	14	3	27	2	24
	4	1967	10.7	62	73	-	46	18	34	43	63
	5	1967	9.0	89	90	-	58	44	50	61	65
Populus deltoides var. occidentalis	1	1968	13.5	87	-	67	-	-	26	-	-
	2	1968	11.3	94	-	28	-	-	0	-	-
	3	1968	11.1	99	-	79	-	-	34	-	-
	4	1968	10.9	80	-	84	-	-	34	-	-
	5	1968	10.9	21	-	7	-	-	0	-	-
	6	1968	10.7	95	-	64	-	-	43	-	-
	7	1968	10.2	97	-	83	-	-	40	-	-
	8	1968	10.2	95	-	92	-	-	55	-	-
	9	1968	10.2	91	-	89	-	-	67	-	-
	10	1968	9.9	96	-	53	-	-	12	-	-
	11	1968	9.7	83	-	72	-	-	28	-	-
	12	1968	9.5	94	-	74	-	-	32	-	-
	13	1968	9.5	98	-	90	-	-	71	-	-
	14	1968	9.5	80	-	43	-	-	3	-	-
	15	1968	8.4	81	-	34	-	-	5	-	-
Populus grandidentata	1	1969	14.8	74	-	-	-	-	-	-	56
	2	1969	13.2	79	-	-	-	-	-	-	65
	3	1969	10.8	70	-	-	-	-	-	-	41

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SUMMARY REPORT

Technical Session 4: Factors affecting seed storage.

Chairperson: S.K. Kamra
Co-chairperson: D.A. Skeates

In his introductory remarks the Chairman, S.K. Kamra, noted that successful storage is the result of many interacting factors. He stressed the importance of the quality of seed at the time of storage, as it is affected by handling and processing of fruits, cones and seeds. Subsequent storage conditions cannot be looked at in isolation but must be considered relative to treatments of seed prior to use.

There were 10 papers in the session. Seven were presented by the authors themselves. Yasuomi Tanaka, Domingo Chai and Jack Pitel were thanked for their assistance in presenting papers by authors unable to attend. Papers dealt with storage of *Abies balsamea*, *Abies* spp. of western Canada, *Betula alleghaniensis*, *Fagus sylvatica*, *Gingko biloba*, *Larix laricina*, *Maesopsis eminii*, *Paulownia tomentosa*, *Picea glauca*, *Picea mariana*, *Pinus banksiana*, *Pinus resinosa*, *Pinus strobus*, *Populus deltoides*, *Populus grandidentata*, *Populus maximowiczii*, *Populus tremula* and *Salix caprea*. Papers represented studies in Canada, France, Japan, Korea, Sweden, Tanzania and the U.S.A. One paper described the evolution of storage conditions in the province of Ontario.

Discussions following the presentation of the papers centred around the following subjects.

1. Terminology. Terms such as germination, viability and emergence are not always used consistently. Terms like inactivity and dormancy may be confusing.

2. Methodology and Reproducibility. Results of various investigations based on different methods for determination of moisture content of seeds. Seed desiccation by sulphuric acid and calcium chloride. Reliability of measurements relative to interacting effects of relative humidity and temperature. Need for seed studies to be continued beyond the germination level, i.e., study of plants as a bio-assay of seed factors. Importance of mechanical and physiological damage to seed as they affect storability and germinability.

3. Temperature. Effect of storage temperatures above and below freezing on retention of seed viability.

4. Light. Interaction of light and dark with auxins and gibberellins as they affect germination and dormancy.

5. Moisture. Effects of drying seed following stratification on subsequent germination.

6. Genetics. Relationship between seed storage duration and genetic and physiological processes during subsequent seed germination and seedling development.

7. Application to field conditions. Need for parallel field and laboratory tests.

8. Biochemical activity and Physiology. Numerous subjects related to seed and plant chemistry and a need to understand physiological processes. Effect of storage conditions on after-ripening. Effect of alternate successive warm and cold treatments of seed on dormancy. Effect of optimum and critical moisture contents in seed storage in relation to temperature and methods of attaining water content. Synchronization of germination with selective water absorption techniques. Effect of variation in growth inhibiting and growth stimulating substances on germination. The need was expressed for dialogue between seed researchers, biochemists and physiologists to help seed people understand mechanisms involved in seed physiological and biochemical reactions.

9. Tropical species. The greatest need for information is on the lesser known tropical species. Here, improved research facilities and training of seed scientists and technicians are needed. All information is of value even if studies are of different standards of sophistication.

The session led to considerable debate and valuable exchange of knowledge.

WORKING PARTY RESEARCH REPORTS

EXCURSION REPORTS

BANQUET SPEECH

CLOSING SPEECH

LIST OF PARTICIPANTS

WORKING PARTY RESEARCH PROJECT REPORTS

The ten projects initiated in Bergen, Norway, in 1973 and the four projects initiated in Yamanaka Lake, Japan were briefly reviewed and discussed. Projects 1, 3, and 12 are now completed. The project 13 has been altered to "Germination ecology" and the new project will be coordinated by Mr. Sahlén (Sweden). A possible new project "Biochemistry of tree seed," to be coordinated by Mr. Pitel is described later under New Projects. The following notes summarize the meeting discussion.

Project No. 1: Survey of Equipment, Supplies and Methods in Forest Tree Seed Work (Coordinator: F.T. Bonner).

The project, initiated in Bergen, Norway, was completed with USDA publication Forest Service General Technical Report SO-13.

Project No. 2: X-ray Group (Coordinator: S.K. Kamra).

In an international IUFRO-ISTA cooperative project, in which 17 laboratories of the world participated, the X-ray radiography was examined as a new possible analysis method in seed testing. The report was presented at the 1980 ISTA Congress at Vienna, Austria (Simak & Sahlén).

Project No. 3: World Directory of Tree Seed Workers. (Coordinator: D.G.W. Edwards).

The copies of the world directory published in 1976 are still available at the coordinator's laboratory. It was suggested that announcement about this directory be made in the Newsletters of the other working parties such as tree improvement, physiology and tropical forestry.

Project Nos. 4 and 8: Bibliography, and Code System (Coordinator: M. Simak).

The coordinator explained that after receiving many suggestions from seed workers from different countries, the system is now going to be applied to forest seed literature. The bibliography system has been simplified from the original one proposed at the Yamanaka Symposium.

Project No. 5: Glossary of Forest Tree Seed Terms (Coordinator: F.T. Bonner).

This glossary to be published in three languages (English, French, and German) will only include terms on seed germination. Copies of the draft prepared by the coordinator was handed out to the participants for their comments. The draft will also be circulated in a Newsletter for comments by other members. French and German versions of the glossary will be prepared, through translation, after the English version has been finalized.

Project No. 6: Newsletter (Coordinator: S.K. Kamra).

The Newsletter is now circulated to 450 members. The participants at the Petawawa Symposium not receiving the Newsletter will be included in the mailing list.

Project No. 7: Publication of abstracts of Proceedings of the Petawawa Symposium (Coordinator: Wang and Pitel).

It will be published in the ISTA journal as those of the Bergen, Norway and Yamanaka, Japan Symposia.

Project No. 9: IUFRO-ISTA Cooperation (Coordinator: B.S.P. Wang).

The coordinator reported the amendments on forest tree seed testing adopted by the 19th ISTA Congress in Vienna, Austria. The major modifications include (1) removal of all references to toluene distillation method, (2) removal of the "four times" rules, and (3) introduction of the X-ray radiography test for determination of empty seeds into ISTA prescriptions. A complete report is included in the Working Party Newsletter No. 25. It was suggested that an ISTA-IUFRO joint meeting be held for exchange of viewpoints on the subject of mutual interest.

Project No. 10: Developing Countries Group (Coordinator: S.K. Kamra).

It was pointed out by the chairperson that the working party will have in the future one co-chairperson from a tropical country who will be responsible for this project with the new project name "Tropical Seed Problems." This agreement was made after discussions among the project leaders present at this symposium.

Project No. 11: Publication Board (Coordinator: J.A. Vozzo).

In the absence of the coordinator, the chairperson of the organizing committee announced that the proceedings of the Symposium will be published using the publication guidelines of the Canadian Forestry Service. The chairperson also requested all the participants to assist in editing and correcting the papers presented at the symposium.

Project No. 12: Theory and Practice (Coordinator: Y. Tanaka).

The coordinator briefly reported the results of the survey "Seed Research Needs in U.S." conducted in 1978. The details are reported in Newsletter No. 25. While this project is now completed it was suggested that the working party may consider a future symposium based on the objective of this project in which participants from "theory" and "practice" can meet together.

Project No. 13: Germination of Forest Tree Seed (Coordinator: B.S.P. Wang).

At the request of the coordinator this project was officially terminated and replaced by the project "Germination ecology." The new project will be coordinated by Mr. K. Sahlén Umea, Sweden. The objective is to develop reliable methods for measurements of microecological environments in which seed germinates. It was pointed out that this project would be extremely valuable in improving direct seeding techniques in the field.

Project No. 14: Seed Dormancy (Coordinator: F. Devillez).

In his absence at the Petawawa Symposium, the coordinator wrote to indicate that he has distributed a draft of his viewpoints on tree seed dormancy on two different occasions in recent years. The response has been quite disappointing. In light of the interest expressed by Dr. Sumihiko Asakawa, Tsukuba, Japan the coordinator requested the transfer of responsibilities to him for further development of the project.

New Projects

It was pointed out that the biochemists working with forest tree seed should be organized in their own professional group within the working party. With such an arrangement they can bring discussions to a higher level and at the same time speak to seed workers who are not well informed in biochemistry. The coordinator, J.A. Pitel, announced that a survey of biochemical research will be conducted as a first step in this project. It was suggested that organization of a symposium on biochemistry of tree seed be considered as a future activity. If enough people show interest in this project, then it would be called "Biochemistry of Tree Seed" and become Project No. 15.

Mr. K. Sahlén will form a new Project No. 13 to be called "Germination ecology." A brief description of the objectives of this new project is presented below by him.

"The interest of direct seeding as a reforestation method for *Pinus sylvestris* has increased in Sweden in recent years. The reasons for that are mainly high labour costs with planting and new and improved scarification methods. In connection with that, studies have begun to try to describe the reaction of the seed to the different environmental conditions that occur in the field. The aim of this is to work out sowing methods, which give a good result with a low seed consumption. The environment close to the seed in the field is determined by different abiotic factors, such as temperature, moisture, air humidity, light, oxygen supply etc.

The investigation planned is divided into three steps.

1. Measuring in the field the variation in temperature, soil moisture and air humidity in the surface layer of the ground during the snow-free period.
2. In the laboratory under controlled conditions we will study how the above-mentioned factors influence the seed germination.
3. Results from laboratory experiments will be tested in the field with contemporary measurements of the environmental factors mentioned.

As this is a complicated work, especially as regards to the measuring technique, we would very much appreciate to get into contact with anyone that works with, or has experience of this problem field."

TOUR OF PETAWAWA MANAGEMENT UNIT ALGONQUIN PARK DISTRICT

September 24, 1980

Prepared and conducted by John Wilson
Ontario Ministry of Natural Resources

Welcome to the Algonquin Park portion of your symposium. There are a number of brochures and pamphlets available on the Park history and features, that we hope will be informative. There are also a number of people along with the tour who will try to answer any questions on the Park.

The first stop on the tour is the site of the Spoor Lake Jack Pine Seed Orchard. (Information is available on separate sheets).

The second stop is the Forbes Creek Experiment area. A number of experiments in management of white pine (*P. strobus* L.) and red pine (*P. resinosa* Ait.) have been initiated here from as far back as 1926. The area we will be seeing was an experiment started in 1958, by our research branch. At that time, 15 plots were established (Figure 1) to try various thinning techniques. Basal area data was gathered, cutting took place and the result was recorded. Every five years thereafter, the area was re-measured.

Last year, the area was marked and cut again and appropriate measurements recorded.

Much of the interest in this area lies in its value as a model of the uniform shelterwood system we are trying to implement. The work shows that a pine stand of this type can, if properly cut, put on increased growth in the residual trees, and regenerate itself.

The third stop is in an area that was cut this past spring. This area was laid out as a 3 cut strip shelterwood system in 1968 and first cut in 1968 and 1969. Under that system, the second cut was due but the silvicultural system now has been changed to uniform shelterwood. You will be able to see both systems in a very short walk. Please take note of the amount of new seedlings on the ground.

The fourth stop is at the Achray Work Centre. This is a year round base for timber management and road maintenance. Parks personnel are stationed there only during the Park season (May to September).

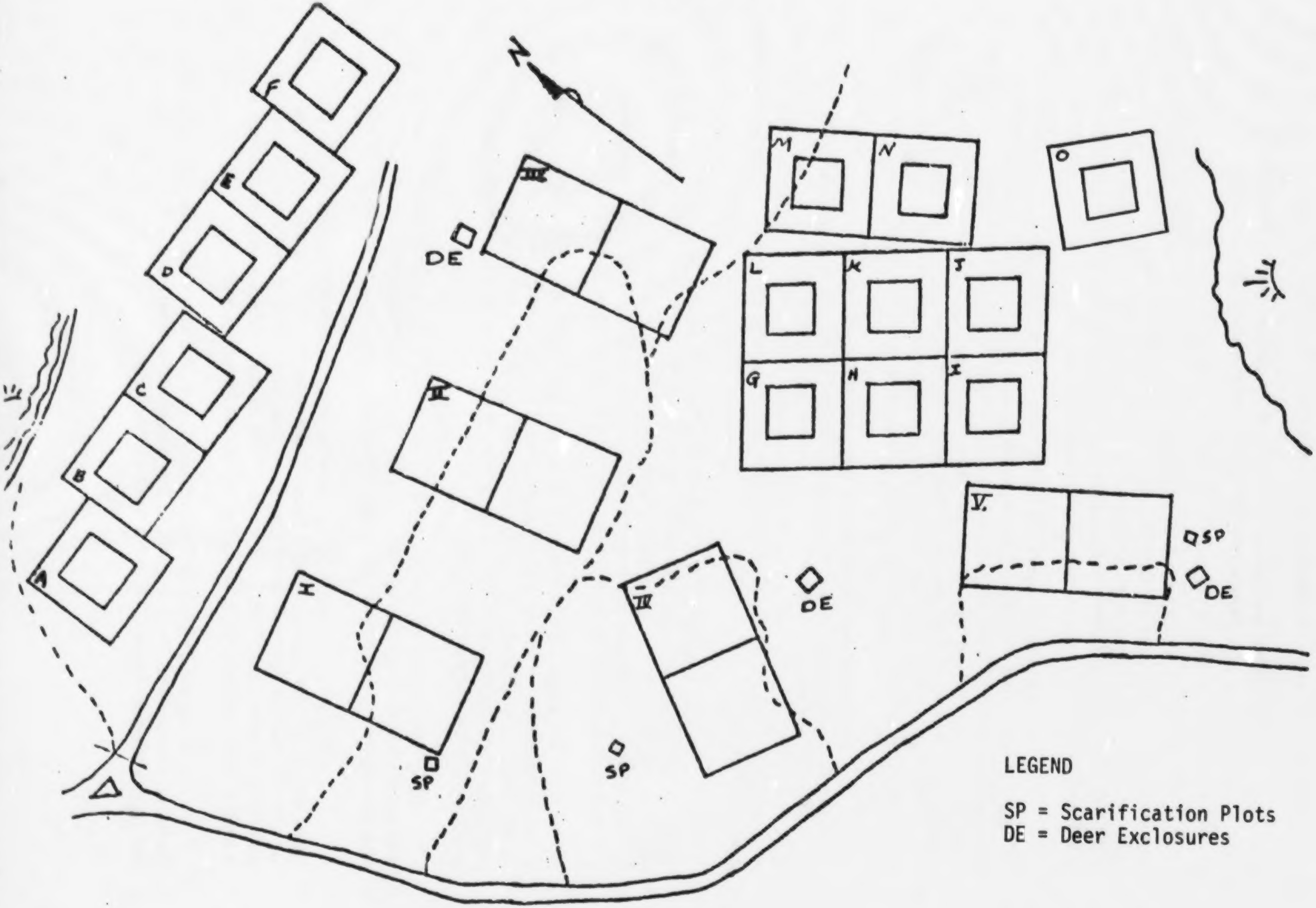
There will be a short session, in the staffhouse basement, by the District Manager and Park Superintendent on Algonquin Park, prior to dinner.

After dinner you will be going to the Barron River Canyon. Handouts are available for this part of the tour. Please heed the warnings about this trail as there are no fences along the canyon wall.

We hope that your time in the Park will be enjoyable and informative. If you want any more information please feel free to contact any of the Park staff on the tour or write to the addresses on the prepared material.

Thank you.

FIGURE 1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SP
LEGEND
SP = Scarification Plots
DE = Deer Exclosures

Forbes Creek Experiment Area

JACK PINE IMPROVEMENT AT SPOOR LAKE, ALGONQUIN PARK

Wednesday, September 24, 1980

Prepared and conducted by C.W. Yeatman and T. Nieman
Petawawa National Forestry Institute

In 1976 this natural jack pine stand of 150 hectares west of Spoor Lake was designated as a seed production area. In collaboration with O.M.N.R. this stand is being used for a practical demonstration of development of a jack pine seedling seed orchard based on plus tree selection, progeny tests and advanced generation breeding.

The stand is 38 years old being regenerated from slash after clear felling and from fire in 1942. Two thinning operations in 1971 and 1972 reduced competition and removed poor quality jack pine by thinning the stand to 2 m x 2 m spacing. The objective was for production of poles. The thinning operations were effective in some parts of the stand but spacing for most of the area is still too dense. The objective of future thinnings is to increase spacing to 4 m to encourage crown growth on the best trees for increased cone bearing capacity.

The stand was divided into two sections by a baseline running parallel to the main road. Control lines were established at 50 m intervals south from the baseline to the main road and north to the edge of Spoor Lake. The lines were spot marked with red paint and a double red band every 50 m was used to indicate a new plot boundary. A total of 394 plots, each approximately 50 m square (0.25 ha) were marked out.

During the fall, 1976, plus tree selection was completed in the area south of the baseline. One plus tree was selected in each plot. Mensurational data i.e., height, DBH, crown width, were recorded at the time of selection. Three to five neighboring trees were measured for comparison. The plus trees were marked with a yellow band and the plot number painted on the tree.

In January 1978, the plus trees were felled and cones and scions collected. In most instances all cones dating back 8 to 10 years were harvested. Fifteen scions were cut, of which 10 were grafted at P.N.F.I. in February 1978. The seed was extracted during the winter and in May 1978 a progeny test of the plus trees was sown in the nursery seedbeds.

The plus trees north of the baseline were selected and felled for cone and scion collection in the winter of 1979. Scions were grafted, seed extracted and a progeny test of these plus trees was sown in the nursery in May 1979.

Presently in the P.N.F.I. nursery there is a progeny test of 306 plus trees which will be planted in the Spoor Lake area in 1981 and also at another site 80 kilometres west of Spoor Lake. A plus tree clonal demonstration will be planted at Spoor Lake (1981) but most of the clonal material will be planted in a breeding arboretum at P.N.F.I. for advanced breeding work.

In spring 1980, seed from Spoor Lake plus trees was sown in Spencer-Lemaire containers. This stock is in the nursery shadehall and will be planted out next spring at Spoor Lake as a seed orchard.

A jack pine seedling seed orchard of Ottawa Valley plus trees was planted at Spoor Lake in June 1980. The origin of the material consists of progeny of plus trees from eight stands in the Ottawa Valley. The seed for this orchard was sown in the nursery seedbeds in 1978 and was planted out as 2-0 stock.

The site of the orchard was originally a logging camp of the 1940's. The entire area was grown up in couch grass with remnants of a few old buildings still standing. The grass was bulldozed into strips running north-south traversing the site at intervals of approximately 40 metres.

A maximum of 30 seedlots were planted in 35 replications. Some replicates were smaller because of poor survival of some seedlots in the nursery. Each seedlot was planted in plots of 6 trees (2 x 3). Spacing between trees within plots was 1 m and between plots 2 m.

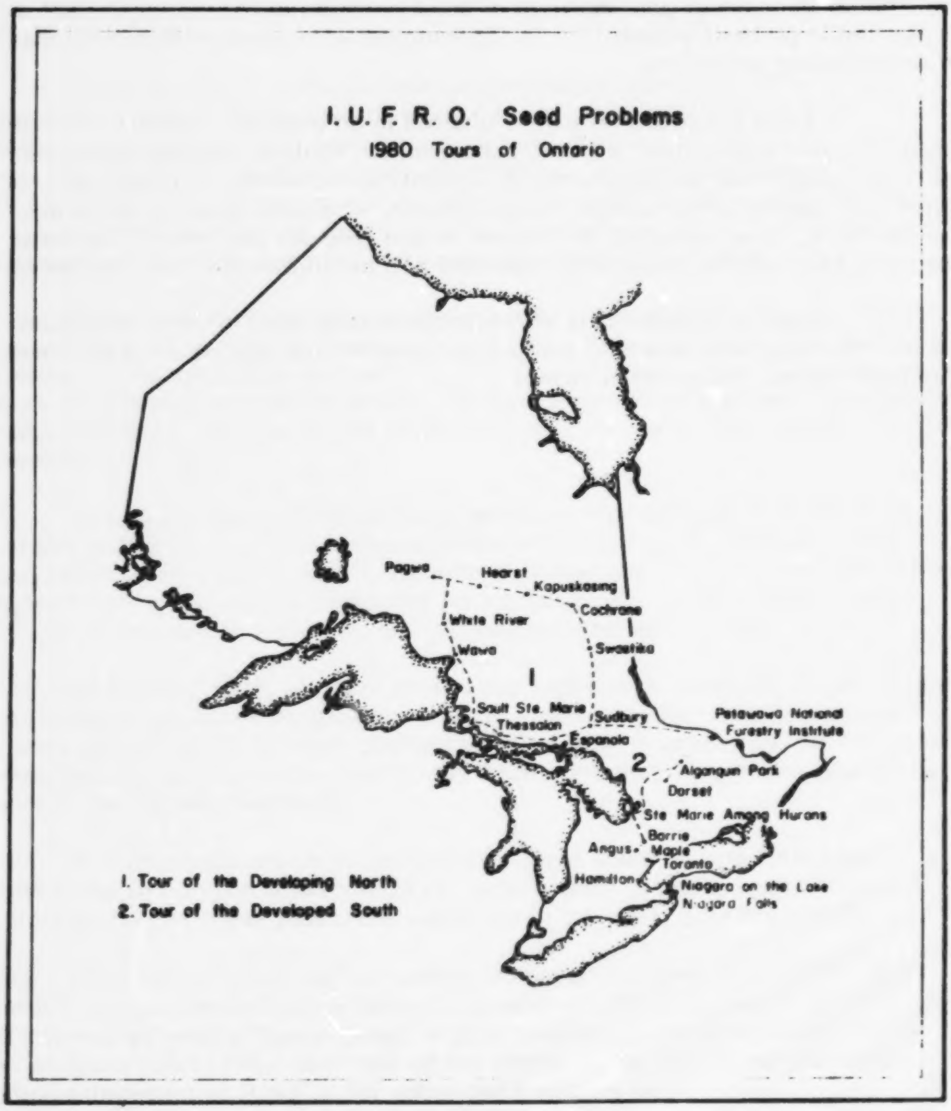
A total of 6,000 trees were planted in an area of 1 hectare. In late August this year a fertilizer with high phosphorous content was applied to each tree to stimulate root development. A high nitrogen content fertilizer will be applied next spring to encourage foliage growth. It is anticipated that by age 10 years the six tree plots will be thinned to one tree per plot and on the basis of progeny test results, the poorest seedlots will be eliminated from the orchard.

Areas for the planting of the progeny test, seed orchard and clone bank in 1981 have been selected and will be cleared this fall. A total of 5 hectares is required for the combined planting.

Touring the 'Land of Opportunity'

D.A. Skeates
Seed Specialist
Ontario Forest Research Centre
Ontario Ministry of Natural Resources
Maple, Ontario

(Map of Ontario)


I. U. F. R. O. Seed Problems
1980 Tours of Ontario
Pagwa
Hearst
Kapuskasing
Cochrane
White River
Wawa
Swastika
1
Sault Ste. Marie
Thessalon
Sudbury
Espanola
Petawawa National Forestry Institute
2
Algonquin Park
Dorset
Ste Marie Among Hurons
Barrie
Angus
Maple
Toronto
Hamilton
Niagara on the Lake
Niagara Falls
1. Tour of the Developing North
2. Tour of the Developed South

Delegates of the IUFRO Working Party on Seed Problems were exposed to a cross section of Ontario. Canadian problems were viewed in a context of Canadian socio-economic conditions. Though Canada is considered an industrial nation, much of her forest is in an early stage of development.

Two tours were conducted in conjunction with the 1980 IUFRO International Symposium on Forest Tree Seed Storage. The first passed through a small portion of the more accessible developing northern Ontario forest. The second provided a visit to the more developed south.

The Developing North

Despite five long days on a bus, the tour provided only a taste of Ontario's northland. Being restricted to highways, delegates received a distorted view of development, through areas largely logged over and past the few scattered towns and villages interrupting the miles of largely uninhabited boreal forest.

The first day was spent passing mainly through agricultural land from Petawawa to Sault Ste. Marie. The area was developed in support of the bustling white pine (*Pinus strobus* L.) logging communities in the late 1800's. With forest lands exploited, a marginal agricultural economy provides the principal support of struggling communities.

From Sault Ste. Marie, the tour travelled northward through the colourful hardwoods of the Great Lakes Region and the beautiful vistas from the rocky hills overlooking Lake Superior. The tourist centres and the extensive Lake Superior Provincial Park attested to the largely recreation based economy of the area.

From Wawa northward and eastwards toward the Ottawa Valley, forestry was the principal land use. Access to some of the areas visited represented only recent development. The small logging communities of Hearst, Kapuskasing and Cochrane gave some indication of the limited population base for sound management of the Boreal Forest.

Finally the tour moved south through mining and agricultural communities of the clay belt. The area visited represents only the more developed fringe of northern Ontario with much of the northern population, and most of the accessible roads and highways. The north is still in the throes of development, the virgin forests still in the first phase of exploitation, the limited population still too inadequate for development of true forest communities. The logging towns and marginal agricultural communities are inadequate to support large scale intensive forest management as one might expect in Europe.

Forestry Problems: The Canadian economy is largely dependent on exploitation and management of these northern resources. The tour visited the Kirkwood plantations near Thessalon. This model of intensive plantation management in the north indicated the potential of forest management for helping to rebuild the prosperity of the agricultural communities across central Ontario. Foresters from Wawa demonstrated the difficulties of creating similar young forests in the shallow stony tills of the pre-Cambrian shield. Clay belt cut-overs near Kapuskasing and Iroquois Falls presented problems of regenerating difficult, wet sites by intensive and extensive silvicultural practices. With little resident population and high labour costs mechanized automated systems are essential.

In underdeveloped areas, protection of forests is limited by inadequate access. Miles of budworm infested forest were observed. Unfortunately delegates had no opportunity to see the vast burned-over areas of Ontario's northwest which destroyed several years of allowable cut. Both problems relate to the excess of mature and over-mature age classes and associated windfall and deterioration. Whereas the most effective answer lies in sound forest management, underdevelopment necessitates highly mechanized protection systems. At Sault Ste. Marie the Ministry of Natural Resources demonstrated aerial fire suppression methods developed in Ontario. Using the world's largest non-military fleet of aircraft, water bombing is used to contain fires until ground crews can be mobilized. The water based aircraft is a major tool for many aspects of forest management in inaccessible forest areas of the north.

Environmental Problems: The tour visited desolated forest lands, destroyed by smelter fumes near Sudbury. Productivity has been eliminated or severely reduced on an estimated 100,000 hectares, and subsequent erosion has exposed large areas of rock. To reduce concentrations of sulphur dioxide, the world's highest smokestack was built. Currently Sudbury is the greatest single contributor to North America's acid rain.

Reclamation work in the form of a wildlife preserve of over 400 ha of revegetated land was visited near the Inco mining operation, an oasis amid the 'desert'. Company officials were most gratified to have a skein of geese fly in right on cue as they finished their presentation.

An interesting greenhouse experiment was part of the Inco tour. Hot air, exhausted from the 7000' level (2100 m) provided even year-round heat for a tomato production operation. Company agriculturists were also experimenting with vegetable crops in warm environments at the 5000 and 7000' levels (1500 and 2400 m) below the surface.

Canadian Forestry Service scientists explained acid rain effects in a small watershed study north of Sault Ste. Marie. Free fall and stem flow were measured in the hardwood forests along with outflows from the watershed. Acidity throughout the system was monitored and mensurational data taken. Vegetation changes were also studied.

Seed Problems: Seed is the first requirement in the silvicultural processes for developing a new forest. Natural regeneration systems, as demonstrated in Kapuskasing depend on reliable seed supplies. Seed tree cuts in white spruce (*Picea glauca* (Moench) Voss) must take irregular periodicity of seed crops into effect. Natural regeneration of black spruce (*Picea mariana* (Mill) B.S.P.) through group seed trees and alternate strip cuts are somewhat less dependent on periodic seed production due to the semi-serotinous nature of the species providing some seed fall between years.

The same problems influence artificial regeneration systems. Direct seeding especially requires large quantities of seed. In particular, prescriptions specify 120-150,000 viable seed per hectare. Again mechanization in cone harvesting appears to be the only answer to acquiring the quantities of seed needed. A modified peat shredder is one device currently in use. A large combine is in the testing stage. Neither was available for demonstration but a jack pine (*Pinus banksiana* Lamb.) cone stripper was demonstrated at Swastika Nursery.

For intensive management, improved quality nursery stock is essential. This necessitates selection and development of permanent seed sources. Though the eventual goal is to collect the bulk of our seed from seed orchards; seed production areas and seed collection reserves represent the province's most important current seed sources. As young natural stands are not generally available, many of these are older, very tall trees. The Arnott Twp. white spruce seed production area south of Hearst is representative of the problem. Here district staff are regenerating this high quality source by both planting and site preparation for natural regeneration. A young white spruce stand at Pagwa in the Geraldton district, regenerated in 1960, was among Ontario's first efforts in stand management for seed production. In Hanlan Twp. one of Ontario's infrequent young stands of white spruce represents the optimum age of production and collection of seed.

Seed collection reserves and seed production areas are being developed as permanent sources of black spruce seed. Reserves are cut for seed and regenerated with the same seed source while seed production areas are managed for collection from selected standing trees. Plantations of selected local origin were visited at the Bonner Centre near Kapuskasing where a cone picking truck-mounted platform was exhibited. A 60' (18 m) mobile tower was also demonstrated by OMNR staff at Cochrane.

Jack pine seed collection reserves were discussed near Swastika Nursery. Large areas have been registered in districts where extensive direct seeding of this species is planned. Annual cuts are planned to meet projected seed requirements.

Planning seed production policy and collection targets have been complicated by changing silvicultural prescriptions. For example, stock production targets of 7-8 million black spruce have been increased to 25-30 million annually. Some districts are also planning to change to extensive management techniques such as direct seeding which will increase seed requirements by 5-10 times.

Seed periodicity, especially in black and white spruce is unpredictable. White spruce tends to produce good crops in some parts of the province each year, but for some areas this means crops every two or three years while other areas experience up to eight years between crops. This results in shortages unless adequate reserves are maintained in storage. No significant black spruce crops have been produced in 5 years through areas visited by the tour.

Periodicity is further confounded by spruce budworm infestations. No spruce crops can be expected in the forseeable future in such areas. Black spruce is not generally considered to be susceptible. Though trees are seldom killed by budworm, a high proportion of flower buds are destroyed or cones severely damaged. In white spruce severe damage was most obvious in the Arnott seed production area. Control studies, demonstrated at the Bonner Centre near Kapuskasing, evoked considerable discussion on environmental problems associated with insect control.

Squirrel predation in jack pine seed collection reserves was discussed in a young stand near Swastika Nursery. Only current year's cones were observed, whereas in older stands several year's serotinous cones were apparent.

Despite adequate overall seed inventories, regional shortages result in seed transfer problems. Potential growth losses due to improper distribution of seed are not fully established. Variable growth performance due to seed source was demonstrated in a Scots pine (*Pinus sylvestris* L.) provenance trial near Espanola and similar variability was discussed in a jack pine provenance study near Swastika Nursery.

Highlights: Each stop on the tour highlighted specific problems of forest management or factors influencing life in the province. Major stops included a visit to Great Lakes Forest Research Centre, hosted by senior Canadian Forestry Service staff. A film was presented, providing an overview of the wide range in disciplines which scientists were studying.

The Bonner Tree Improvement Centre, a former nursery of Spruce Falls Power and Paper Co. has been converted to a study and seed production centre. Plantations in the area were established by the company using super seedlings from seed of selected trees on the licence. Various treatments are planned to increase cone productivity.

Cochrane staff of the Ministry of Natural Resources presented an interesting comprehensive slide show drawing together most of the information observed on the trip, with an emphasis on techniques for regenerating and managing the forests of northern Ontario. Despite staffing restrictions and limited resources, a concerted effort is being made to achieve optimum forest productivity in the north.

Swastika Nursery, one of three major northern stock production centres is working toward its 20 million tree production potential, including bare root and container stock. New greenhouse plans were outlined and techniques for production of accelerated (greenhouse) transplants described. One promising development, the automated sowing of pregerminated seed in peat blocks was demonstrated. The use of waste heat from a gas pipeline pumping station for greenhouse container production was discussed briefly at greenhouses at Ramore north of Swastika.

An overnight stop in a Ministry 'bush' camp provided a unique experience in contrast to the very comfortable accommodation of motels such as the Carolyn Beach at Thessalon, the Wawa Motors Hotel and the Kapuskasing Inn. There were few complaints at the tremendous bush camp meals provided by Ministry staff of the Kirkland Lake and Hearst districts at Burt Lake and Arnott Twp.

Focus: The focus for the northern trip was a banquet held in Kapuskasing. The guest speaker, Mr. A.J. Herridge, Deputy Minister of Northern Affairs, brought the tour into the context of development of a northern society for the benefit of all Canadians. This development must provide good living standards for people of northern communities. Art noted with justifiable satisfaction the improvement in services in communities such as Kapuskasing. Even more satisfying is the improvement in housing standards as more and more people think of their communities as their permanent homes. The north is gradually providing a more satisfying and comfortable lifestyle compared to the rawness of these same towns 10 to 20 years earlier.

With little pressure on land to provide living space, with vast areas of forest land, with a strong recreation base added to established agricultural and mining economies, northern Ontario represents future prosperity in this land of opportunity.

The 'Developed' South

To contrast with the vast and sparsely populated forests of the north, the southern tour was planned to show the interrelation of forest based activities and development of Ontario's southern rural society. It was intended to provide insights into Canadian history and culture as a basis for current Canadian lifestyles. Forestry, a major element in Canada's early development, is still an essential element for healthy growth in the 80's and represents a potentially powerful component of future prosperity.

The tour started in the marginal agricultural landscapes of the Madawaska Valley. It cut through a portion of Algonquin Park where a forest economy has gradually changed to a recreation base. The hardwood forest of the Canadian shield were only starting to turn colour, showing some of the potential beauty for which Canada is famous. The tour passed between the lakes of Muskoka, the heart of our water based recreation lands and on to the glacial moraines where large scale forest plantations have been developed and the alluvial plains, the agricultural heartland of Ontario. In these agricultural areas are centred the provincial tree seed plant, stock production nurseries and provincial research facilities. Beyond the urban centres of Ontario, delegates visited horticultural stations, a fruit farm in the controversial Niagara fruitlands, finally reaching Canada's premier tourist attraction, Niagara Falls.

Development of Canadian Society: Central Ontario's history is synonymous with the exploitation of the white pine. From the Ottawa Valley to Dorset, delegates passed through the aftermath of this exploitation, sleepy communities once thriving with the harvest of red (*Pinus resinosa* Ait.) and white pine logs. Algonquin Park was created long after the loggers had cut the biggest and best pines for masts and the square timber trade. This facet of Canadian history was fitted together with the history of colonization and the impact of the white man in Indian culture at Saint Marie among the Hurons near Midland. The reconstructed Jesuit mssion provided an excellent portrayal of life and its hardships for early Canadians. Early Canadian life and landscapes were also illustrated in the paintings of the Group of Seven exhibited at the McMichael Collection in Kleinburg. Both facilities were developed and are operated by the Ontario Ministry of Culture and Recreation.

The 1980 traveller of these historical lands must be aware of the dramatic changes in forest land use. At Dorset, the tour was treated to a slide presentation of the recreational activities around the lakes and trails of the Algonquin region. The Leslie Frost Centre of the Ontario Ministry of Natural Resources is dedicated to creating ecological awareness particularly amongst children and teachers in our educational system. The development of resources to meet the recreational needs of Canadians and their international guests was even more apparent in provincial parks. A stop at the museum in Algonquin Park, a lunch time visit to Springwater Park at Midhurst and a tour through the Niagara Parklands demonstrated the emphasis on recreation in Canadian life.

Forestry Development in the South: While some forest lands have been converted to recreational use large areas of forest, cleared for agricultural purposes had to be abandoned as unsuitable for viable farming. Much of this still lies in marginal farming areas, some reverting to brush or low quality forest. The tour passed through intensively managed young red pine plantations of Simcoe County established on the sandy soils of glacial moraines. County owned forest land is managed by the province under long term agreements.

Similar agreements have been drawn up between the province and private land-owners in order to reforest lands unsuitable for agriculture or to improve the quality and yield of existing forests. One of such properties was visited near Welland where local Ministry staff discussed management of the deciduous forests along Lake Erie. Seeds of 28 of the southern species were distributed. The problems of trying to create and maintain productive forests on private lands were discussed with delegates.

The Ontario Tree Seed Plant was the single most important visit on the tour. Here almost all the seed for the provincial reforestation program is processed. Cones are shipped from as far away as Kenora in northwestern Ontario, distances of up to 1800 km. Cones and fruits are stored in screened drying sheds until processed. In the extraction building cones are put into rotating screened drums in kilns heated largely by burning the waste products of the extraction process. Much of the seed cleaning equipment in use had been developed at the plant. The final product, cleaned seed of 75 species of trees and shrubs are stored under frozen conditions, if necessary for many years before shipment to the field or to nurseries for production of stock, for the diverse needs of forest management on crown and private lands of Ontario.

Two of the major stock production centres in Ontario were visited. Delegates toured Midhurst Nursery which provides stock for reforestation in both southern and northern Ontario. Of particular interest was the large stock of several species produced for aesthetic purposes along highways, in parks and other provincial institutions. A few delegates stayed an extra day to visit Orono nursery. A propagation project with black and white spruce has resulted in a system for producing numerous trees from each viable seed. The system is intended primarily for large scale reforestation from the limited amount of improved seed available from young seed orchards.

Two provincial forest research facilities were visited. Provincial research is centred at the Ontario Forest Research Centre at Maple north of Toronto. Field stations are located at Thunder Bay, Sault Ste. Marie, Dorset and the Stock Production research unit near Barrie.

Two of the OFRC programs were described at Maple. In the Tree Breeding Unit emphasis was placed on hybrid studies in hard pines and poplar, the use of isoenzyme technology in jack pine population genetics, flower induction and resultant seed set and propagation of conifers by cuttings. The hardwood nursery on the station provided a basis for discussing wet land forest rehabilitation following losses due to the Dutch Elm disease. A research program relating to farm/forest products such as honey and nuts as forest food products was discussed. Near Barrie the effects of various stock production and field planting practices on plantation performance was outlined.

Related Horticultural Operations: The tour visited the Royal Botanical Gardens in Hamilton. The principal stops were the hardwood arboretum, the famed rock gardens and a tour of the propagation facilities. Techniques used with forest tree species and the wide variety of species represented were of particular interest.

The School of Horticulture was visited near Queenston. As guests of the Niagara Parks Commission we were given a thorough tour of the park, the training ground for Canada's future recreation personnel. The beautifully manicured gardens were a first stop followed by a visit to the greenhouses and propagation facilities.

Highlights: For seed scientists, undoubtedly the most important stop on the tour was the Ontario Tree Seed Plant already noted. The Angus plant is the nerve centre for processing almost all of the province's seed needs. The Ontario Forest Research Centre, with its genetics, hardwood, and seed research programs were also highly relevant to seed specialists.

From a tourist point of view, Niagara Falls takes top billing for visitors to Ontario. With the Niagara Parks Commission staff hosting the tour it was most unfortunate we could not allocate the time to enjoy fully the many experiences they had to offer in Niagara. A beautifully warm, sunny day and a southerly breeze, blowing mists away from the falls, contributed to an excellent view of Canada's best known tourist attraction.

A major focus for the Ontario tours was provided at a banquet in Barrie hosted by the Hon. James A.C. Auld, Minister of Natural Resources in Ontario. Mr. Auld, speaking of Ontario's contribution to world forestry, outlined problems of developing a forest resource in a land still developing its basic transportation network and building remote communities as bases for sound resource management. Seed problems were basic to the whole silvicultural program and steps being undertaken to solve these problems were noted. Mr. Auld welcomed the seed scientists to Ontario and hoped that their visit gave them a better understanding of our problems. He also was confident that their knowledge and experience would contribute greatly to the federal and provincial scientists working together to improve Ontario's capabilities in finding solutions.

Appreciation

Dr. Milan Simak, as Chairman of the International Working Party on Seed Problems, expressed the feeling of the delegates when he thanked Mr. Auld for his contribution to the unqualified success of the symposium. In particular the minister's assistance with transportation made the two tours possible. But even more important Dr. Simak noted the enthusiastic reception provided by foresters and technicians of Mr. Auld's ministry in each and every district we passed through. Despite weekends, the rain (for 5 consecutive days of the northern tour), minor delays along the way, etc., ministry staff were there waiting at each stop to explain what they were trying to achieve and demonstrating how they were doing it. Federal and provincial scientists accompanied the tour, sharing their experience over many years of current Ontario forest history. Their participation and that of the field staff, along with Mr. Auld's personal interest and support had made this visit a most interesting, enjoyable and enlightening experience.

BANQUET SPEECH by

The Honourable James A.C. Auld on September 29, 1980
Ontario Minister of Natural Resources

I am very glad to have this opportunity to talk to so many distinguished visitors as yourselves who have come here from so many parts of the world.

It is our good fortune that all the areas you have visited and have still to see are in our own province of Ontario. Because the Ontario Ministry of Natural Resources has the honour to be your hosts for this evening's banquet, I have come to bring you especially warm greetings from the Government of Ontario.

Nearly three hundred years ago, an English writer called Jonathan Swift said "Whoever could make two ears of corn grow upon a spot of ground where only one grew before, would deserve better of mankind than the whole race of politicians put together." Perhaps he would judge all of us who work in legislatures as harshly today, but I hope not.

In modern times, politicians are fully aware of the vital contribution that seed scientists can make to the needs of their own people, and to the rest of the world. We honor you, and see to it that you have the facilities you require to pursue your studies fruitfully.

As I expect you have already realized from talking to members of my ministry's staff the Ontario Ministry of Natural Resources has to accept wide responsibilities in this province in addition to forest management and silvicultural research.

They include equally broad management responsibility for Ontario's mineral resources, and its wildlife, with its game and fish populations, as well as management of nearly 100 million hectares of public land and water in areas that are non-urban and non-agricultural, and more than 100 provincial parks and recreation areas.

As you may know from your travels, some governments prefer to divide responsibility for these apparently quite different activities between two, three or more separate departments. As a matter of fact, that is what is done by our friends in the Federal Government of Canada. Both methods have their advantages and disadvantages.

For our part, we have found that often the first solution that is proposed to answer a problem in one of our areas of concern will create an unexpected problem in another. In other words, the two activities are not so entirely separate as they had seemed to be.

A new tree-harvesting method, for instance, may harm the habitat of a dwindling herd of game already suffering the effects of a bitter winter. Or potentially valuable mineral deposits may exist in an area where it has been considered that a recreational facility should be developed.

Once these conflicts are known, it usually proves possible to make alternative plans. We try to avoid conflicts before they arise by arranging for all our different specialists to work with knowledge of the work being done by their colleagues in other branches of our ministry.

However, if you wonder whether being answerable for so many activities is likely to create an unusual number of difficult questions everyday, you are quite right!

The difficult questions can cover anything from forest fires to uranium mining to shortages of speckled trout to the prices to be charged for overnight camping in our parks. It all helps to make this the most interesting and varied job I have had in my life.

This month you have seen the sheer magnitude of our forests. They provide one of our province's most valuable natural resources. We recognize that they represent a heritage we are fortunate to possess, and a responsibility to be guarded for succeeding generations. We know as well that part of each year's harvest is required to meet the needs of the world outside Canada.

Last year our forests yielded a cut of 18 million cubic metres with a stumpage value of $46 million. That was almost a quarter of Canada's total forest production for the year. More than 30 per cent of our forest production, to a total of $1.9 billion, was sold to export markets last year, most of it pulp and newsprint.

The regional significance of our forest industries is great. They provide 29 per cent of all manufacturing employment in the northeastern areas you visited, and no less than 77 per cent in northwestern Ontario.

In total, 78,000 people in Ontario are directly employed in forest industries, and a similar number of our people have work indirectly related to forest production.

Our lands are used for more than the value of their forest products, though. Last year, they provided hunting vacations for 30,000 visitors from outside the province as well as nearly 600,000 residents; and good fishing for 650,000 residents in addition to many thousands of visitors. Our provincial parks welcomed in all no less than 9.5 million visitors in that year.

So we have good reasons to recognize how fortunate we are in the natural resources of Ontario. They have made it possible for us to afford to devote a considerable amount of care, study and research to the best ways to maintain, develop and manage these resources.

Our good fortune creates another responsibility, too: The responsibility to share whatever knowledge and expertise we have gained with other countries who might benefit from our experience - just as we benefit from the knowledge and experience of others.

We are not alone in this, of course. The same response to the world's needs is forthcoming across Canada. I am, however, particularly proud of the part played by members of my ministry's staff in helping the development of natural resources in many African, Asian and South American countries.

Those countries are at present less developed than our own. Nevertheless, we cannot consider our own province of Ontario by any means fully developed yet. Those of you who took the tour of Northern Ontario before your workshop will comprehend why I say that. Some of the areas you travelled through have only become accessible in the past 20 years by major highway.

There are other areas of the province previously only known to a few prospectors and trappers, and perhaps the most adventurous hunters, where roadbuilding is only now being undertaken.

Rather more than 20 million hectares of forestland have still to show satisfactory evidence of being in even the earliest stage of forest management. The vastness of our forestlands is unquestionably a gigantic asset. As you may guess, though, it can also be the source of gigantic headaches.

The distances involved make roadbuilding itself a major undertaking in northern Ontario. Some of it is built by the companies harvesting the forests, some by my ministry. All told, more than 21,000 kilometres of forest-access road have been built, and are being regularly maintained. That is not much less than the total length of our provincial highway system.

We know now how much care must be given to the health of our forests. The cost of development, and the heavy expenses of regeneration, and the heavy expenses of regeneration programs are recognized as essential even in times, like the present, of tight fiscal constraint. As a result, the budget approved by the Ontario legislature for silviculture has risen from $20 million in 1972 to its present level of $70 million.

We have made great progress since the first important steps to regenerate forestlands in northern Ontario were taken in the 1950s. Nevertheless, out of 195,000 hectares cut annually, we are as yet only able to treat 80,000.

We can expect natural regeneration to occur satisfactorily in another 60,000 hectares, but that leaves about 55,000 hectares in need of attention. So we cannot consider that we are more than halfway to achieving our objective.

Closing the gap between our present performance and our objective will not be easy, but it will become much easier by improvements in our seed technology.

As members of the international working party on seed problems, your specialized knowledge will have given you a rapid understanding of the types of problems our own specialists have to overcome, even if those problems differ somewhat from those with which you are better acquainted. You have also followed the steps they have taken in their search for the most effective solutions.

Where natural regeneration methods are to be applied, the problem of periodicity in seed supply is only one that has to be met. Techniques for improved direct seeding have also to be found.

Here the challenges include the development of appropriate technology and equipment, since very large quantities of seed are necessary. Furthermore, for some species the collection period is relatively short, and the manpower limited.

As yet, we cannot safely predict what yield of seed we will get from clonal seed orchards or our permanent seed-collection areas. We still have to learn effective ways to collect seed from standing trees in those seed-collection areas. We must also discover appropriate techniques for obtaining the optimum yield and quality, and to minimize loss to insect infestation.

Because of their economic value, the northern conifers that I have been speaking about absorb 90 per cent of our total silvicultural budget. Another different set of seed problems constantly demanding our specialists' attention is connected with the numerous hardwood species found in southern Ontario. Our hardwood seed program is smaller, and that makes the diversity of the problems appear even greater.

Happily, we having willing allies. I am very gratified by the spirit of co-operation existing between my ministry and the staff of the Canadian Forest Service, which forms part of our federal government. That co-operation is particularly marked between the seed specialists in the Canadian Forest Service, some of whom are travelling with you, and their opposite numbers in my ministry. It is on a level of personal friendship, since I have found that people who work with nature nearly always share the same dedication to their work.

As an illustration, I think that you will be interested to hear that Ray Latimer, whom you met this afternoon, has devoted all his working life to my ministry's tree seed plant at Angus. He became its superintendent, in fact, when his father retired from that position. Now Ray Latimer himself is retiring in November, this year.

Between them, the Latimer family has served the Angus tree seed plant since 1923. I think you will agree that its smooth-running efficiency and the friendly helpfulness of its staff provide a tribute to the Latimers, and to their dedication.

Tomorrow you will visit our Ontario forest research centre at Maple. There, just as at all the other places you have visited, you will see evidence of the strong communication links between our federal and our provincial scientists and foresters.

We also gain much benefit from the visits we received from scientists like yourselves from other countries. Your questions stimulate the minds of our own specialists, and the information you can provide enriches their knowledge. I sincerely hope you have similarly gained something of equal value to your own work from being here.

Beside that, though, I hope you have personally enjoyed the time you have spent in our province. I realize that you have had a busy program to occupy you, and I doubt whether you have had much time for anything beyond your special field of study.

There is so much to enjoy in Ontario, however, in our countryside, our rivers and lakes, and in our large cities that I wish that at least some of you may be able to extend your visits so as to experience the warmth of welcome you will find from everyone.

If that is impossible - in fact, even it it is possible, as well - I trust you will all try to return to Ontario another year. We will always be happy to see you.

CLOSING SPEECH by the Working Party Chairman, Professor M. Simak (Sweden)

Ladies and gentlemen.

We have now reached the point when this symposium on Seed Storage has to be closed. During the next few hours we are starting our travel home in different directions, filled with many new impressions, enriched knowledge and fresh ideas for further work. We have met at this symposium many colleagues, some of whom we will remain in touch with in the future. These are some of the great values which this symposium has brought us.

We all enjoyed listening to the papers dealing with storage problems of forest tree seeds from different theoretical and practical aspects. The lively discussions, kept at high level, often had to be curtailed by the session chairmen because of the time factor. This is the best proof for the great engagement of all the participants. A great deal of the success of this symposium is due to the chairmen of the different sessions, and we all would like to thank them heartily for their good work.

We are all very grateful to our friend Ben Wang and all the members of the Organizing Committee for the excellent preparation of the 4th Symposium of our W.P. Especially, we appreciate the thorough organization of the excursions, during which we became familiar not only with the Canadian forestry but also with the Canadian hospitality. We express special thanks to Doug Skeates, our hard working and informative leader during the presymposium excursion. Our last request to the Organization Committee is: please extend our sincere gratitude to all the organizers and persons who contributed to this successful symposium, including the excursions.

At this symposium two new projects were initiated: the biochemical aspects in seed research and the ecology of seed germination. Both projects will open new valuable work fields.

Moreover, we have decided that the position of the co-chairman of our W.P. in the next mandatory period should be reserved for a seed worker from some tropical country. We hope in this way to activate the necessary seed research in the tropical countries.

During the closing banquet we had an opportunity to listen to an interesting lecture about "Nuclear energy and the environment" presented by a scientist from the Chalk River Nuclear Research Laboratory. Indeed, nuclear energy represents a tremendous power. However, we should remember that a proportionally more powerful and creative energy is hidden in every little living seed with which we are working. It is our task to use this power carefully for establishing new and better forests - a goal which we had in the inscription for the theme of this symposium.

Herewith I close this 4th Symposium of the W.P. on Seed problems and thank you all for your excellent cooperation.

LIST OF PARTICIPANTS

ACKERMAN, Francine
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, Canada
K0J 1J0

ALTMANN, Dick
Pine Ridge Forest Nursery
Box 750
Smoky Lake, Alberta, Canada
T0A 3C0

ANDERSON, Moe
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, Canada
K0J 1J0

BAKER, Bill
Ontario Forest Research Centre
Ontario Ministry of Natural Resources
Maple, Ontario, Canada

BOWDEN-GREEN, Rob
Ministry of Forests
Duncan Seed Centre
Box 816
Duncan, British Columbia, Canada
V9L 3Y2

CAMPBELL, Mark
Box 90
Wabigoon, Ontario, Canada
P0V 2W0

CARPENTER, Stanley B.
Dept. of Forestry
University of Kentucky
Lexington, Kentucky 40546
U.S.A.

CHAI, Domingo
Forest Research Centre
P.O. Box 1407
Sandakan, Sabah
Malaysia

CORRIVEAU, Armand
Centre de Recherche Forestiere
des Laurentides
1080 Rte du Vallon, C.P. 3800
Ste.-Foy, Quebec, Canada
G1V 4C7

EDWARDS, George
Pacific Forest Research Centre
506 West Burnside Road
Victoria, British Columbia, Canada
V8Z 1M5

FOGAL, Willard
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, Canada
K0J 1J0

GILCHRIST, Ernie
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, Canada
K0J 1J0

HAAVISTO, Fred
Great Lakes Forest Research Centre
Canadian Forestry Service
P.O. Box 490
Sault Ste. Marie, Ontario, Canada
P6A 5M7

HADDON, Brian
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, Canada
K0J 1J0

KAMRA, Krishan
Department of Genetics and Plant
Physiology
Swedish University of Agricultural
Sciences, Faculty of Forestry
S-90183, Umeå, Sweden

LAMONTAGNE, Yves
Ministere Energie et Ressources
Service de la Restauration
200 B, Chemin Ste-Foy
4ieme Etage
Quebec, Canada
G1R 4X7

LATIMER, Ray
Ontario Tree Seed Plant
Box 70
Angus, Ontario, Canada
L0M 1B0

LEADEM, Carole
Research Branch
B.C. Ministry of Forests
1450 Government Street
Victoria, British Columbia, Canada
V8W 3E7

MULLER, Claudine
Centre National de Recherches
Forestieres
Laboratoire D'Analyses de Graines
Champenoux 54280 Seichamps
France

PITEL, Jack
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, Canada
K0J 1J0

PRASAD, Raj
Forest Pest Management Institute
Canadian Forestry Service
Environment Canada
P.O. Box 490
Sault Ste. Marie, Ontario, Canada
P6A 5M7

RAUTER, Rose Marie
Room 3631, Whitney Block
Queen's Park
Toronto, Ontario, Canada
M7A 1W3

ROBERTSON, Sandy
Canadian Forestry Service
Nfld. Forest Research Centre
Box 6028
St. John's, Newfoundland, Canada
A1C 5X6

SCHEER, Gary
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, Canada
K0J 1J0

SCHOOLEY, Hugh
Nfld. Forest Research Centre
Canadian Forestry Service
P.O. Box 6028
St. John's, Newfoundland, Canada
A1C 5X6

SIMAK, Milan
The Swedish University of
Agricultural Sciences
Faculty of Forestry
S-90183, Umeå, Sweden

SKEATES, Doug
Ontario Ministry of Natural Resources
Ontario Forest Research Centre
Maple, Ontario, Canada
L0H 1E0

SMITH, Ron
Maritimes Forest Research Centre
P.O. Box 4000
Fredericton, New Brunswick, Canada
E3B 5P7

STRAKA, Alena
Provincial Tree Nursery
R.R. #6
Edmonton, Alberta, Canada
T5B 4K3

SUSZKA, Boleslaw
Institute of Dendrology
Kornik, Poland

SWAILE, Brian
Ontario Tree Seed Plant
Box 70
Angus, Ontario, Canada
L0M 1B0

TANAKA, Yasuomi
Weyerhaeuser Company
P.O. Box 420
Centralia, Washington 98531
U.S.A.

TRAVNICK, Dal
Forest Pest Management Institute
Canadian Forestry Service
Environment Canada
P.O. Box 490
Sault Ste. Marie, Ontario, Canada
P6A 5M7

TROWSDALE, Walter
Nursery Superintendent
Pineland Forest Nursery
Hadashville, Manitoba, Canada
R0E 0X0

VANBORRENDAM, Wim
Forest Research Centre
Ontario Ministry of Natural Resources
Maple, Ontario, Canada
L0J 1E0

WANG, Ben
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, Canada
K0J 1J0

WINSTON, David
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, Canada
K0J 1J0

YEATMAN, Kit
Petawawa National Forestry Institute
Canadian Forestry Service
Chalk River, Ontario, Canada
K0J 1J0